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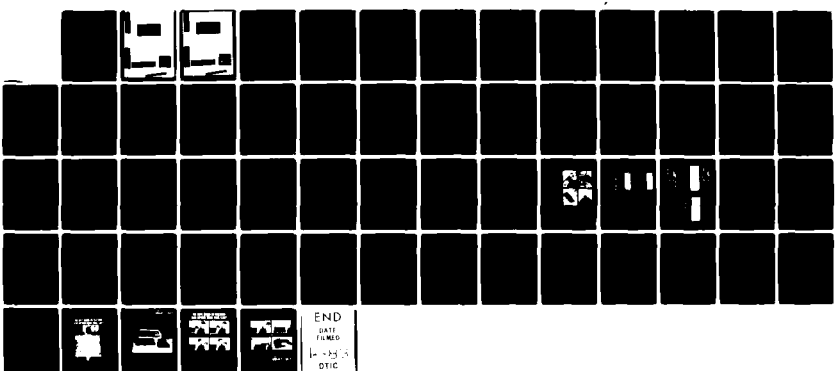

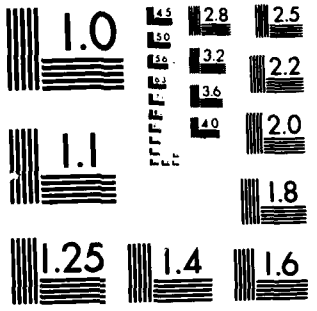

Defence and Civil Institute of Environmental Medicine

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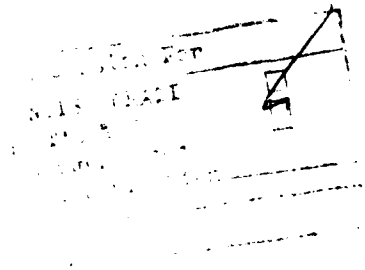
ABSTRACT

A review of current drug of abuse screening methods available in Canada in 1982 is presented. These methods include classical thin-layer chromatography (TLC), the commercially available Toxi-Lab® TLC system, immunoassay procedures such as enzyme multiplied immunoassay technique (EMIT®), and radioimmunoassay (RIA - Abuscreen®). Advantages, disadvantages and limitations of each method, and costs of equipping and production are compared. Also reviewed are confirmatory procedures used to validate positives found by the less specific screening methods and these include gas liquid chromatography (GLC), gas chromatography-mass spectrometry (GC/MS), and high pressure liquid chromatography (HPLC).



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INTRODUCTION

The large increase in drug abuse and numbers of drugs which have been added to the list of abused drugs over the last two decades have created demands for laboratory methods suitable for detecting drugs in biological fluids. Many methods have been developed and published, based on a wide variety of analytical techniques ranging from classical thin-layer chromatography (TLC) to gas chromatography combined with mass spectrometry (GC/MS). The bulk of published methodology usually deals with analysis of a specific drug or class of drug, sometimes including metabolite detection. Considerably fewer reports deal with drug screening on a comprehensive basis and these arise from toxicology and forensic laboratories (13,17,67) or institutes of drug abuse research (10).

Non-invasive techniques apply almost solely to urine screening. Other body effluents which have been suggested for screening purposes include breath, saliva, and sweat. With the exception of breath analysis for alcohol there exist no other widely used, clinically-proven drug screening methods applied to these latter samples. Expired alveolar air will reflect the concentrations of compounds in the pulmonary capillary circulation which have sufficiently high vapour pressures at physiological temperatures (22). Some drugs such as amphetamines, chloral hydrate and methadone, may be suitable candidates for breath analytical technique development, but the majority of drugs and their metabolites have very low vapour pressures. Their concentrations in alveolar or end-tidal air would be below sensitivity levels of most if not all the current analytical technologies. Breath analysis therefore would not be suitable for more comprehensive drug screening.

The salivary excretion of various drugs has been studied extensively in recent years. Many investigators have observed that drug concentrations in saliva are often proportional to concentrations in plasma. There are indications that, for many drugs, the salivary level is equal to the free or protein-unbound concentration in plasma (18). For the therapeutic monitoring of blood levels it has been suggested that saliva could substitute for plasma and much of the published work deals with the relationship of saliva to plasma ratios for individual drugs administered chronically over long periods of time. Levels present are consequently relatively high. After single dosing, drug levels in saliva can quickly drop below detectable quantities. This is the result of protein binding of the drug and its metabolites, saliva reflecting only the free plasma drug component. Morphine, for example, is detectable in saliva for up to six hours only after a single therapeutic dose (67). There may be an advantage, however, to screening saliva in addition to another body fluid such as urine. A positive saliva test may indicate more recent contact with a drug than would be indicated by positive presence of drug metabolites in urine which often persist for days or even weeks

after ingestion of some drugs. Urine still remains the sample of choice for a non-invasive measure of drug use and most existing methodology has been developed for and applied to urinalysis. With minor adaptations, however, most methods can be applied to other biological fluids.

PHARMACOLOGICAL CONSIDERATIONS

Results of drug tests must be interpreted in the light of some knowledge of the drug's metabolism and pharmacokinetics. Drugs are taken in by various routes, orally, by inhalation, or by i.v. or s.c. injection, and similarly excreted via several routes, kidneys, G.I. tract, lungs, mouth and skin. For practical purposes the kidney provides the principal route of excretion. Whatever the route of entry into the body, the drug is carried by the bloodstream throughout the body and metabolism and excretion occur simultaneously thereafter. Urine tests are designed to detect the drug and/or major metabolites. In some cases the ingested drug is essentially completely metabolized, or biotransformed, within the body to form other usually chemically related compounds, and it is only these metabolic products which are present in the urine. In most other instances varying proportions of the drug may be excreted unchanged along with metabolites. It is necessary to know in what form or forms each drug of interest will be present and what the capabilities of an individual analytical test are in differentiating parent drug from metabolites. Examples of drugs of abuse which are completely metabolized before elimination by the kidney are heroin, diazepam, tetrahydrocannabinol, and cocaine (about 90%). Analytical procedures designed to be specific for the unmetabolized form of these drugs only will not be suitable for urinalysis. It is also important to recognize that some drugs have metabolites in common. Morphine present in urine may be due to ingestion of morphine itself, or heroin, or codeine, and on the basis of the presence of morphine alone, the actual drug ingested cannot be known.

DEFINITIONS

Concentration

Drug concentrations in urine are usually expressed in micrograms (μg, 10^{-6} grams) or nanograms (ng, 10^{-9} grams) per millilitre (ml). Each analytical method for a drug or class of drug has a range of concentration for which it is suitable, and a minimum concentration which it can detect. This point will be discussed further when considering individual methodologies and drugs. Practical ranges for drug detection by current analytical procedures are shown graphically in Figure 1.

A number of factors affect the concentration of a drug or metabolite in urine. In general, concentration decreases as time

since ingestion lengthens. However, a low level of drug in the urine may signify either a small dose taken recently or a much larger dose taken days previously. The volume of urine excreted over a specified period of time also affects the resultant concentration. A high rate of diuresis will dilute the excreted compounds perhaps to the point where the amount per aliquot analyzed falls below the sensitivity level of the particular test being applied, resulting in a false negative. A further factor determining drug concentration is the metabolism of the individual which affects drug biotransformation and excretion rate, and wide interindividual variations occur here.

Limit of Detection

The limit of detection or sensitivity of an analysis for a particular compound refers to the threshold level of detectability. It is defined as the minimum amount or concentration that can be detected reliably (10,11). In immunoassay methods [radioimmunoassay, (RIA), and enzyme multiplied immunoassay technique (EMIT)] sensitivity is determined by finding the lowest calibration point (concentration) that differs significantly from the blank. The level of significance chosen is the 95% confidence limits. This level is selected statistically from the amount of scatter or deviation of the blank and experimental values about their respective means. It is the lowest concentration (i.e., highest sensitivity) that will minimize false negatives and yet not be so low that false positive results are common.

For some methods it is often difficult to state the sensitivity with any degree of precision. Such methods include thin-layer chromatography (TLC), gas chromatography (GC), and GC-mass spectrometry (GC/MS). This is due to problems inherent in methodology from laboratory to laboratory. Extensive and variable losses, for example, may be incurred in sample preparation, high background from other endogenous compounds may mask drugs, instrument detectors lose sensitivity with age or dirt accumulation, etc. In such cases ranges of minimal detectable amounts are usually quoted. The most efficient and experienced laboratories may expect to achieve results at the lower end of these ranges.

Specificity

This is the degree to which a test can discriminate between closely related drugs and metabolites. Lack of specificity can be a drawback and result in false positives where related drugs have different actions. It can also be an advantage when metabolites are detected as well as the parent drug, since the sensitivity is thereby increased. The least specific of the common assays are the immunoassays since they are usually class specific, reacting with various affinities to many related drugs of a class and their metabolites. The most specific method is combined GC/MS and this is often the

reference method recommended for validation of results achieved using other tests (29).

Precision

Precision is the variation between replicate measurements (11,20), reported usually as the relative standard deviation or, in percentage terms, the coefficient of variation (c.v. = 100 x s.d./mean). Repetitive analyses of any sample will generate this information but, as precision is concentration-dependent, the concentration should be stated. As the level of drug approaches the limit of detection of the method the c.v. increases.

Accuracy

Accuracy refers to the difference between the experimentally determined mean and that value which is accepted as the true value of the quantity measured (20,24). This is not a very useful term since in chemical analyses the so-called "true" value is represented by the experimentally determined mean of replicate analyses of a known quantity of the compound. Some meaningful measure of accuracy of a method might be obtained by comparing mean values with those obtained using a more specific analytical procedure, if such is available. A more useful measure of accuracy suggested by de Ridder (20) is to run spiked or control samples containing known amounts of the drug of interest. The percent deviation from the "true" or original reading then comprises a running record of the method's accuracy over time. This is, however, more a record of long term day-to-day precision of a method.

Validity or Reliability

Validity of a method includes components of both sensitivity and specificity and refers to the ability of the test to reliably detect a drug or its metabolites in biological fluids (10). This can really only be assessed by examining known populations of users and non-users to measure the occurrence frequencies of true and false positives and true and false negatives using a format such as the following table:

	Drug Taken	
Test Result	Yes	No
Positive	True positive	False positive
Negative	False negative	True negative

The ideal case is nil false positives, and nil false negatives. The more sensitive and specific a test is the lower the incidence of false positives and negatives will be, and, the more reliable the procedure in differentiating drug use from non-use. A less sensitive technique will not detect low levels of drug or metabolite and false

negatives would be expected. Similarly, a less specific method which does not differentiate compounds with similar chemical structures, will result in a higher incidence of false positives. The less specific a test the greater is the necessity that positive results be confirmed by another method or methods, and preferably one with greater specificity.

GENERAL ANALYTICAL CONSIDERATIONS

Most procedures for drug analysis in urine require preliminary sample cleanup to separate drugs and their metabolites from the many other endogenous compounds which could interfere with final readings. Such preliminary purifications commonly involve organic solvent extraction, ion exchange, or adsorption-desorption from a non-ionic solid support, or some combination of these (46,66,73). All of these procedures involve some loss of drug and none are perfect in that some interfering background material is always carried through the extraction steps. In general, the cleaner the final extract the lower the recovery will be for the drug or drugs of interest. There is always a compromise between recovery and degree of cleanup. Immunochemical techniques, on the other hand, have a great advantage over other procedures in that they do not generally require prior purification. Pretreatment losses are avoided, and as one consequence, immunoassays are more rapid and much simpler to perform.

SCREENING METHODS FOR URINE ANALYSIS

A. Chromatographic Procedures

1. Thin-Layer Chromatography (TLC)

TLC for drugs is a standard screening method available in most laboratories where drug analyses are conducted routinely. There is no universally accepted procedure and methods are many and varied (43,53,54,73). The urine sample must be cleaned up before it can be chromatographed successfully. It may also require either enzyme or acid hydrolysis to convert the glucuronide- or sulphate-complexed drugs and their metabolites to free drug. This step can enhance considerably the recovery of those drugs which are excreted mainly in water-soluble complexes. In general, a minimum of two extractions are required, one at an acid pH and the other a basic pH in order to maximize drug recoveries. Methods using a single extraction at some neutral pH have not proven satisfactory as judged by overall drug recoveries (66).

The final urine extract in a small quantity of organic solvent is spotted close to one edge of the thin-layer plate. Drug standards are spotted adjacent to the samples. The plate is then "developed" in a closed tank or container by allowing a solvent, called the developing solvent, to pass from the bottom edge by capillary action

to some point near the upper edge. As the solvent moves through the urine extract spot the compounds present are carried up the plate at different rates. The degree to which a component moves from the point of application is determined by its relative solubility in the solvent system used and the adsorptive forces exerted by the silica gel coating of the thin layer plate on the compound. In this manner preliminary separation of extracted urinary constituents is effected. The final detection step involves spraying the plate with chemicals that react with specific chemical groups contained within the drugs. The drug is identified both on the basis of distance travelled from the origin to the distance travelled by the solvent front, this value being called the Rf, and on the colour reaction or reactions produced by the sprays used.

There are numerous solvent systems in use and even more visualization systems. Each laboratory must determine from the choices available which combination of procedures bests suits its own individual requirements and then set up a TLC laboratory with necessary equipment purchased from chromatographic specialty supply houses. For a total screen using TLC a minimum of two plates, and often three or four, run in different solvents is usual. This reduces the number of sprays used sequentially per plate and decreases the chances of lower sensitivity through inadvertent over-spraying of a visualizing agent. Usual detection systems found in practice would include:

for amphetamines, ninhydrin followed by exposure to UV light;
for barbiturates, mercuric sulfate followed by diphenylcarbazone; and
for opiate narcotics, iodoplatinate and Dragendorff's reagent.

The chief advantages of the classical TLC drug screen are found in the low cost per sample analysis compared to other procedures. Also many drugs are screened for simultaneously and, even with the time consuming spotting process, a technician should complete 50 to 60 complete drug screens in a normal working day. It must be strongly emphasized, however, that TLC is a screening procedure only. Positive identification for most drugs cannot be conclusively made on the basis of a single Rf value and one or two colour reactions (43). Because the chromogenic spray reagents used react with certain chemical groups they are therefore not drug specific but will react with any compound possessing that particular chemical configuration. Alternate methods must be employed to verify suspected positives found by TLC procedures.

2. Toxi-Lab System

There is available on the market currently a much simplified and standardized TLC broad drug screening system called "Toxi-Lab". This kit is manufactured by Marion/Analytical Systems in the United

States and marketed in Canada by Western Scientific (see Annex A). Toxi-Lab has simplified the extraction-spotting procedures so that the organic extract from urine is evaporated onto a cut-out spot from a plate, specially impregnated paper strips in this case. After evaporation is complete the "spot", or disc, is then inserted into the TLC strip ("toxi-gram") along with a disc or discs containing various mixtures of drug standards. These discs are prepared and supplied by the manufacturer which also alleviates the problem of obtaining drug of abuse standards as is required for conventional TLC methods. For a total drug screen, two extracts are prepared and two chromatograms run, Toxi-Lab "A" for basic and neutral drugs, and Toxi-Lab "B" for the acidic drug class. Instead of spraying the developed TLC strips, they are dipped sequentially directly into the chromogenic reagents to induce colour development. Spots observed at each stage are described on the result sheets by Rf value plus a description of the spot's colour at each of the dipping steps. Identification is then made by comparison to the supplied drug characteristics table in conjunction with photographs of sample chromatgrams of positive urines. Over one hundred drugs including metabolites are now included in the colour guides and supporting documentation.

The system is capable of identifying all common drugs of abuse except cannabinoids, lysergic acid diethylamide (LSD), and psilocybin ("magic mushrooms"). Also benzodiazepine metabolites may not be seen at therapeutic levels unless a preliminary enzyme hydrolysis step is carried out to allow sufficient extraction of free, unconjugated metabolites.

The chief advantages of Toxi-Lab are speed and simplicity and an increase in the number of samples which may be processed per day as compared to conventional TLC methods. As with all TLC detection procedures the reliability of the method depends almost entirely on the experience of the personnel in interpreting plates. Extracts are relatively dirty leading to streaking problems on the chromatograms. The more concentrated the urine sample the worse this streaking is, although the problem can be minimized by reducing the volume of urine extracted from the five mls recommended by the manufacturer. Plate interpretation is very subjective in nature and it is only by acquiring sufficient background experience through running known positives that the technician can become familiar with TLC patterns associated with the individual drugs. This is a distinct disadvantage in a small hospital laboratory situation where volume is low and few drugs encountered and technicians largely untrained and inexperienced in drug of abuse analysis.

The Toxi-Lab system is appearing in large hospital laboratories and methadone clinics where the emphasis is on monitoring patients for compliance in prescribed therapeutic drug use or in suspected drug overdose. In large volume laboratories involved in

forensic analyses or clinical treatment such as the Ontario Centre for Forensic Science and the Addiction Research Foundation, Toxi-Lab is now being considered as an adjunct to, but will probably never replace, conventional TLC facilities built up over time and backed up by years of experience.

B. Immunoassay Techniques

These comprise the second type of drug screening system currently available. Included under this classification are EMIT (enzyme multiplied immunoassay technique, Syva), RIA (Abuscreen-radioimmunoassays, Roche), LF (Latex flocculation, Abuscreen-Agglutex, Roche) and HI (haemagglutination inhibition, Technam, Inc.). Of these, only EMIT and RIA are presently marketed in Canada. Roche-Diagnostic Agglutex will be available in late 1982 for the detection of opiates and barbiturates only. The Technam-HI kits were distributed by BDH Chemicals but have not been supplied since the late 1970's. They too were limited to assays for morphine, barbiturates, and methadone.

Three of these systems, namely EMIT-st (single test), Agglutex and Technam-HI were the subject of an evaluation report by the U.S. Naval Drug Screening Laboratory (38) in 1981 as potential portable urinalysis systems for use by the Department of Defense (see Annex B for a copy of the final conclusions of the report). All these assays are based on the same immunochemical principles. Antiserum is produced which contains specific antibodies against a particular drug (antigen) and is one of the critical reagents for the assay. The second critical reagent is labelled or tagged drug. This is a drug which is complexed either to a specific enzyme in the case of EMIT, or rendered radioactive by the introduction of tritium or attachment of iodine-125 in the case of RIA, or coupled to a latex particle as with Roche-Agglutex.

An immunoassay is carried out by starting with a preset amount of both antibody and labelled antigen. A small amount of urine which may or may not contain any of the drug (unlabelled antigen) is added. If any drug is present in the urine, the result is a dilution of the labelled antigen. The antigen-antibody reaction is allowed to proceed for a set period of time according to the method and then a measure of the unused or unreacted labelled antigen is made. The amount of labelled antigen remaining at the end of this reacion time is a proportional measure of the amount of drug present in the urine sample. Major differences between immunoassay techniques lie in the means by which the unreacted labelled drug is determined. EMIT measures a by-product of an enzyme-substrate reaction photometrically. RIA measures the free radiolabelled drug using either a gamma counter (for gamma-emitting isotopes) or a liquid scintillation counter (for beta-emitting isotopes). The latex flocculation test requires a subjective visual measurement of the degree of

agglutination observed. Maximum agglutination or flocculation occurs through the antigen-antibody reaction in the absence of free drug. Any free drug reduces the amount of latex-antigen participating in the reaction, in effect inhibiting flocculation. Therefore a reduction in agglutination indicates presence of free drug.

1. EMIT-dau Assay (EMIT-Drug Abuse Urine Assays)

The first EMIT-dau kits were introduced by Syva Company for morphine assays ten years ago (62,64). Since then the number of drug of abuse assays produced by Syva has expanded to include amphetamines, barbiturates, methadone, propoxyphene, cocaine metabolites, phencyclidine (5,19,33,47,50), benzodiazepines (9,57) and, most recently, cannabinoids (14,59,69).

The principal advantages of EMIT-dau techniques are ease of sample handling, rapidity of analysis, and sensitivity. No sample pretreatment or extraction and cleanup are necessary as is required for all chromatographic procedures. The assay is performed at 30° to 37°C using a simple spectrophotometer with a thermally regulated microflow cell. Syva markets the required instrumentation for EMIT-dau as the EMIT/Lab 5000 Instrument System. An automatic programmable timer-printer is used to record the result, which is a change in absorbance (OD). Also part of the initial equipment is an automatic diluter for sampling 50 µl of urine and 250 µl prepared buffer. All necessary reagents and drug calibrators are supplied in individual kits along with complete stepwise instructions for their use. A single analysis is complete in less than 2 min with result read and recorded automatically. The analysis is semi-quantitative with the concentration of the drug in the urine determined by comparison with known spiked samples or calibrators.

A disadvantage of the EMIT system is that one can screen for only one drug at a time. If all nine drugs or drug classes are screened for, nine separate analytical runs must be performed. Each run requires its own standards or calibrators, and the cost is multiplied accordingly. Another disadvantage, mentioned previously, is the decreased specificity of the analysis as compared to chromatographic techniques. Immunoassays are class-specific as opposed to drug-specific assays, and may cross-react with chemically similar compounds. They do not, for example, differentiate between the various barbiturates as TLC and gas-liquid chromatography (GLC) do, but record a total barbiturate concentration. Because of their inherent cross-reactivity EMIT-dau assays are subject to a 3 to 5% incidence of false positives (2). For this reason the manufacturer stresses that any EMIT-dau positive must be confirmed by another analytical procedure (5,14,69). As a screening procedure, however, the lower specificity can provide a distinct advantage over other procedures in that the sensitivity of the anlysis is greatly increased. Not only is the parent drug detected but its metabolites,

both free and conjugated forms, are reactive, and the additive effect can extend the time post-ingestion that the drug may be detected. Many drugs are detected by TLC for only up to 18-30 hours after intake, while immunoassays in general allow detection for several days to more than a week after a single dose. They are ideal, therefore, for epidemiological surveys of the incidence of individual drug use/abuse in large populations.

A number of investigators have compared EMIT assays to other analytical techniques such as TLC, RIA, GLC or GC/MS (25,33,50,51, 52). While not as sensitive as RIA for most drugs, EMIT assays are often equally as sensitive as GC/MS procedures and usually much more sensitive than TLC. The minimal technical skills required to perform the tests plus the rapid results obtained objectively make the EMIT system ideal for mass screening purposes. EMIT-dau has been introduced into penal and other correctional institutes in the U.S. as an in-house replacement for now very costly analyses formerly provided by commercial laboratories (3). RCMP laboratories in Canada also use EMIT-dau for cannabinoids (55) and benzodiazepines specifically. Many medical centres and toxicology laboratories have introduced the EMIT system into their integrated drug screening and research programs (25,52,71).

2. EMIT-st (Single Test) Assay

In 1981 Syva Co. introduced the EMIT-st assay system which is a simplified version of the EMIT-dau system designed for portability and rapid, qualitative results (Annex C). Tests are available currently for the same nine drug classes as with EMIT-dau. The single test system is one of the three portable drug detection systems evaluated for potential use by the U.S. Department of Defense (38), and it is the one deemed most suitable over the other two tested as an adjunct to their present bimodal (GLC/RIA) DoD laboratory system. Major reasons for this choice were:

- objectivity of data interpretation,
- speed of analysis, less than 2 min,
- minimal security required during analysis time, and
- built in safety features against operator error.

All the reagents necessary for the test are contained in dried form in an assay vial. These include antibody, coenzyme nicotinamide adenine dinucleotide (NAD), enzyme-labelled drug, substrate for the enzyme, and buffer. Reconstitution occurs upon addition of a small amount of urine plus 3 mls of distilled or deionized water. Two vials must be run simultaneously, one with urine, the other with the reference calibrator containing a known amount of the drug being tested for. These vials are shaken briefly, placed in a spectrophotometer cell, and in 90 sec the instrument prints out either a positive (+) or a negative (-) result. A positive indicates that the

unknown contains more drug than the calibrator. The sensitivity of EMIT-st assays is similar to or better than those achieved by EMIT-dau except for cannabinoids. The cutoff level for EMIT-dau cannabinoid detection is 20 ng/ml while for the EMIT-st it is 100 ng/ml, essentially five times less sensitive. This lack of sensitivity is a serious drawback and many samples, even during the first 24 hrs post-smoking, may be read as negative for cannabinoids using this assay (45).

The EMIT-st is still too new for independent critical evaluations to appear in the literature although a Syva study (19) covering five of the tests indicate excellent agreement with corresponding EMIT-dau analyses and three of the assays meet DoD requirements sufficiently (38).

3. Radioimmunoassay - Abuscreen (RIA)

RIA for drug of abuse screening first appeared commercially in 1972 and Hoffman-La Roche has been a leader in their development. Kits are available in Canada now for morphine, barbiturates, amphetamine, cocaine metabolite, and phencyclidine and, later in 1982, a kit for cannabinoids will be introduced. Iodine-125 is the labelling isotope used and therefore a gamma scintillation counter is required for measurement of radioactive isotope levels. As with other immunoassays, Abuscreen RIA is based on the competitive binding to antibody of tagged antigen and unlabelled or free antigen. The free drug displaces labelled drug from the limited antibody present. After precipitation and centrifugation the supernatant fluid, which contains the unreacted antigen, is transferred to tubes for counting in a gamma counter. A positive urine is identified qualitatively when the radioactivity is greater than that of a positive calibrator, or quantitatively by comparison to a standard curve. RIA is generally the most sensitive detection method available, often into the nanogram per ml (ng/ml) range. It too is subject to cross-reactivity. Positive results are confirmed by other methods such as GLC or GC/MS where available for validation and to rule out the possiblility of interfering cross-reactive compounds. There have been many studies comparing RIA assays to other methodologies. It is used widely for opiates, barbiturates, amphetamine and cocaine (13,15,30,50,51,58, 67), as well as methadone (33), methaqualone (7,8), and more recently for phencyclidine (35) and cannabinoids (6,52).

RIA has been accepted as a first line screening method in many large drug detection facilities such as USAF and USN drug laboratories and the Ontario Centre for Forensic Science (13,38,67,68) for a number of years. There are still, however, a limited number of drugs of abuse for which kits have been produced. For this reason it usually forms only one of a battery of different methodologies employed by such laboratories.

The advantages of RIA are, of course, its sensitivity in screening as well as its suitability for automation where thousands of samples may be processed in a week (68). The disadvantages lie in the initial cost of equipping a laboratory with a relatively expensive gamma counter and a large volume swing-head type centrifuge. There is the added problem of handling and disposal of radioactive wastes generated and the need for monitoring, regulation, and regular inspection according to the local laws governing the use of radioactive materials and the facilities. It is necessary to have technicians who are trained in the proper use of radioisotopes and in the operation and maintenance of scintillation counters.

CONFIRMATORY METHODS

All of the preceeding screening assays require confirmation of positive findings. This is certainly true for medico-legal or forensic purposes where the outcome of court litigation may depend upon the reliability of a drug assay (1,10,26,29,76). This section will outline some techniques and instrumentation used for validation of suspected positives. Confirmatory methods are all based on chromatographic techniques because the separating power of these procedures provides the greatest specificity in compound identification.

A. Gas-Liquid Chromatography (GLC)

GLC has been a standard method used in many laboratories and may often have been the only method of analysis employed (10). As with TLC there is no universally accepted procedure but all samples must be cleaned up to extract, isolate, and concentrate any drugs as free of urinary impurities as possible. Enzyme hydrolysis to free complexed drugs and metabolites is usually a prerequisite in order to maximize recoveries.

In GLC the separation of compounds in an extract is similar in principal to that of TLC but the physicochemical means is substantially different. A small amount of extract, one or a few uls, is injected onto a heated column where the components volatilize immediately. They are carried by means of a carrier gas flow, helium usually, through the column. The column is packed with a small particle size inert support coated with a liquid, or, in capillary chromatography, with a wall coating of liquid. The components partition between the gas and liquid phases according to their relative affinities for each and progress through the column at different rates. The time to reach the end of the column is characteristic for a particular compound and is referred to as the retention time (RT). By this means individual drugs are usually separated from each other, and from their metabolites as well, before reaching the detector. For this reason GLC techniques are considered the most specific of all screening methods.

The conventional detector is the flame ionization detector (FID). Other detectors such as electron capture (EC), nitrogen-phosphorus (NPD), or mass spectrometer (MS) have greater sensitivity than the FID. Each time a compound strikes the detector the signal is recorded graphically. Identification is achieved by comparing the retention time of the unknown compound to that of an authentic standard run separately under exactly the same conditions.

A major requirement for successful GLC is that the compound be relatively volatile at temperatures below the degradation temperature of the column packing. Unfortunately many drugs and their metabolites are not sufficiently volatile and must be derivatized to increase their volatilty. Many derivatizing agents are employed to achieve this, among them diazomethane which is unfortunately toxic and potentially explosive (43). Silyl ether formation or perfluoroalkylation are now commonly used derivatizing procedures. Any of these methods adds an additional step and increases the chances of loss during the already extensive pretreatment required. Spiked samples containing known amounts of drugs being screened for must be carried through the entire extractive-derivatization process to ensure that overall recovery is adequate and to enable accurate quantification.

Unfortunately there is no single GLC analysis that will cover the whole range of drugs during one chromatographic run. The literature presents a multitude of methods the very least of which involves two columns (1,4,23,27,28,56). The same column should not be used for both acidic and basic drugs since salt formation and precipitation resulting from acid-base reactions will shortly ruin the column. Many of the published techniques are directed at the limited screening of homogeneous chemical classes of compounds and at the detection of one or a few substances (26). Each drug or class of drug requires different extraction procedures to ensure maximum recovery, so that a single urine sample may have to undergo multiple extractions at differing pH's with different extracting solvent systems or solid adsorbents to achieve anything approaching a comprehensive drug screen. Where many laboratories formerly used GLC as a primary screening technique, it is now often used more as a confirmatory procedure for suspected positives found by other simpler and faster methods (8,17,25,26,33,49,67,74).

The advantages of GLC are its combined specificity and sensitivity. While less sensitive than RIA and other immunoassays, it will separate and identify the different drugs within a class and their metabolites. False positives are usually less than 1% with GLC when the technique is properly carried out (10). Under such conditions quantification of drugs may be made to below 100 ng/ml with an accuracy of ±5%. The disadvantages of GLC are the time-consuming preparation of samples and standards, small number of samples which can be processed, initial cost of equipment, and the high level of

expertise required in personnel maintaining the equipment and interpreting the data.

B. Gas Chromatography-Mass Spectrometry (GC/MS)

The combination of gas chromatography with mass spectrometry provides the most specific and sensitive method currently available for the determination of drugs and their metabolites in complex biological mixtures (29). It may often be the only method able to validate results from other sensitive but less specific screening techniques such as immunoassays. The preceeding section of GLC applies here as well for sample preparation and chromatographic separation of the components of a biological extract. The high specificity and sensitivity of the method are due to the MS detector.

When a compound exiting from the GC column enters the MS source it is ionized by one of several means, usually electron impact (EI) or chemical ionization (CI). In EI ionization the compound is ionized by a beam of electrons and usually fragments into many different ionized molecules. The analyzer mass filter separates the individual ions present on the basis of their mass, and the electron multiplier amplifies and counts ion signals which are recorded electronically. For any compound the ionization pattern, or mass-to-charge ratios (m/z) of the fragment ions, is specific and reproducible under set conditions for MS parameters, such as temperature and electron voltage.

In CI the compound is mixed with charged reactant gas ions in the source and ionized through ion-molecule reactions, which usually involves the addition or transfer of a positively charged hydrogen ion to the substrate molecule. This is a more gentle form of ionization than EI and may result in only one significant ionized species, i.e., the parent molecule with its molecular weight (mw) increased by one mass unit. This is most useful in determining the mw of the unknown, but somewhat less useful for positive identification unless other characteristic fragment ions are formed in sufficient quantity.

Identification of the compound is made on the basis of displaying the same retention on the column plus the identical mass spectral ionization pattern as that obtained for a known standard. The chances of two different compounds having both the same chromatographic RT and mass spectrum is extremely remote. To increase sensitivity, which is necessary to detect many drugs at therapeutic levels, the technique of selected ion monitoring (SIM) is used (48). This involves limiting the scan to a few characteristic fragment ions instead of the wide range of masses scanned in normal mode. These ions must be present at the right RT and in the correct proportions to provide positive identification of the presence of a compound. Using SIM subnanogram amounts of drugs often may be identified and quantified with variations less than 10% in a few mls of urine. Full

scan mode requires at least 10 ng of most substances to obtain a complete spectrum (78).

GC/MS has been used since the mid-70's for pharmacokinetic studies (48), and is widely used by larger, well-supported forensic laboratories (13,29,71,75) in specific drug screening applications as the reference method and for quality control of other methods (25). The major deterrents to widespread use of GC/MS are the extremely high costs of the instrumentation and its maintenance, the inherent complexity of the technique, and the high level of training and skill required for its effective use (29). Because of its complexity and slow throughput GC/MS is not suitable for large volume screening.

C. High Pressure Liquid Chromatography (HPLC)

One of the most recent chromatogaphic techniques to be applied to drug analysis is liquid chromatography carried out under pressure flow at room temperature or very slightly elevated temperatures. This technique allows the simultaneous analysis of basic, acidic, and neutral compounds which is a distinct advantage over the gas chromatographic methods in use. The low temperatures used reduce the possibility of pyrolytic degradation to which some drugs are susceptible. Because of the relative newness of the technique, a limited amount of literature has appeared on its application in toxicological analysis (31,32,34,41). It is essentially a separation technique similar to gas chromatography but with certain advantages such as minimal sample preparation with no derivatization of compounds necessary. In fact, a number of papers report direct injection of body fluids onto HPLC pre-columns (39,61).

Detection sensitivities using the U.V. absorption HPLC detector vary widely but usually drugs cannot be detected in amounts less than 1 ug. Electrochemical detection can lower the limits of detection into the ng per ml range (37), but the most promising combination for increased sensitivity and specificity is the integrated LC/MS system (36). This may well supplant current GC/MS methodology in providing future reference methods for detection of drugs and their metabolites in biological samples. Once again the major drawback for smaller clinical laboratories will be the initial cost of the system and the requirement for highly skilled technical support staff.

COMPARATIVE SENSITIVITY OF SCREENING METHODS

Table 1 lists the detection limits claimed and, in the case of immunoassays, the cut-off levels recommended by the manufacturer. While it may appear that TLC sensitivity approaches that of the immunoassays in many instances, it must be remembered that these TLC values are for individual drugs while immunoassay levels represent the total of cross-reactive materials present which includes

metabolites as well as parent drug.

In general terms TLC is capable of detecting most drugs in urine up to 24 to 30 hours after a moderate dose. An exception is the conventional TLC method for benzodiazepines in which these drugs are first acid hydrolyzed to their corresponding benzophenones. TLC sprays can detect benzophenones up to 3 to 4 weeks after a single therapeutic dose (42,44). This kind of sensitivity is the exception rather than the rule for TLC, however. Of the screening methods RIA is the most sensitive. The USAF Drug Detection Branch which uses RIA for its initial drug screen estimates the following durations for positive findings for the following drugs:

Amphetamines	up to 7 days
Opiates - heroin	up to 4 days
- codeine	up to 1-3 days
- morphine	up to 4 days
Cocaine	up to 4 days
Barbiturates - short-acting	up to 1-3 days
- intermediate	up to 3-5 days
- long-acting	up to 4 weeks

With other immunoassays, which are less sensitive than RIA, somewhat shorter durations post-ingestion for positive readings may be expected. EMIT-dau for cannabinoids, however, is reported to indicate presence of cannabinoids in urine for 4 to 10 days following smoking (69).

Confirmatory methods of GLC, GC/MS, and HPLC vary widely in reported sensitivities for many drugs, due mainly to variable recoveries achieved by sample preparation techniques employed for cleanup. An experienced laboratory could expect limits of detection approaching those reported for RIA methods.

COST COMPARISON OF METHODS FOR DRUG ANALYSIS

Table 2 indicates the initial cost for purchase of equipment required to set up a drug screening facility for each of five screening methods. Operating costs included in the table represent 1982 prices for individual reagents and kits. The least expensive is TLC while the portable EMIT-st system is the most expensive with a cost of $26.00 for a complete screen of nine drugs per sample. Table 3 lists ranges of initial equipment costs for the various column chromatographic methods. The material operating costs for these techniques is relatively low due to the limited number of samples which can be processed in a day, but maintenance costs could be considerable.

CONCLUSIONS AND RECOMMENDATIONS

Suitability for Hospital or Base Laboratories

Of the five screening methods included in this report those most suitable for the small laboratory where experienced personnel may not always be available are the enzyme immunoassays, either EMIT-dau or EMIT-st. The major reason is the objective nature of the data interpretation which places the onus on an instrument to print out a positive or a negative result. All other methods require certain degrees of skill and experience on the part of the technical personnel involved, either in the handling of radioactive materials and associated equipment or in the interpretation of chromatographic plates. A second reason is the range of abused drugs covered by EMIT. While very restricted when compared to TLC, EMIT does currently have kits for most of the major abused drugs and, of primary concern, for cannabinoids which are not detected using Toxi-Lab and not yet available in RIA-Abuscreen. The EMIT-dau system, while initially more costly, is half the expense of EMIT-st in daily production costs. It is also currently much more sensitive for cannabinoid screening than the single test. Unless there is a requirement for portability of drug screening facilities the EMIT-dau is recommended over the EMIT-st. All manufacturing companies offer good support and continuing consultation for their systems. The immunoassay techniques will definitely be expanded through continuing research and development to provide additional drug analyses in the future.

Any suspected positive found using screening techniques must be validated by another method which employs different analytical principles, or is more specific. For the small laboratory not possessing such capability, this means sending out all samples with positive findings to another laboratory for confirmation. Even with a verified positive urine the most that can be concluded from urine analysis is the following, "With specific, sensitive, and reliable methods it may be possible to say, with almost absolute certainty, that the subject had, at some time in the recent past, taken some of the drug in question" (43). For epidemiological studies or many law enforcement purposes this information may be all that is required. Results of urine analyses are not amenable to answering further questions such as how much of the drug was taken and how long ago was it taken.

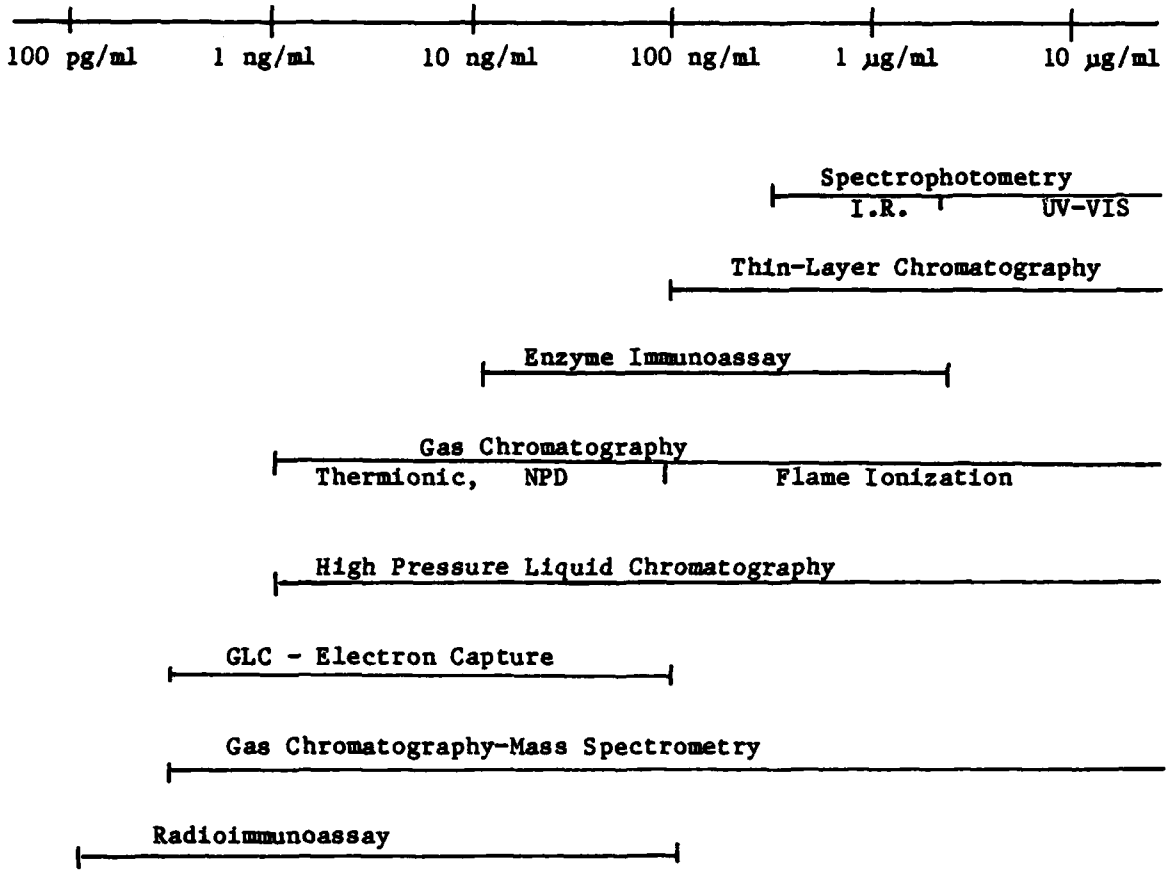

Figure 1. Practical Range of Usefulness of Analytical Techniques Against Drug Concentration in Biological Fluids.
(Adapted from Reference 21)

Table 1. Comparative Limits of Detection for Screening Methods (1)

Drug	TLC	Immunoassays		
	Toxi-Lab (5 ml)	EMIT-dau (50 µl)	EMIT-st (50-100 µl)	Abuscreen (100 µl)
Opiates	1.0 µg/ml	0.5 µg/ml	0.5 µg/ml	40 ng/ml
(Morphine)		0.3 µg/ml	0.3 µg/ml	0.5 ng/ml
Amphetamine	0.5 µg/ml	2.0 µg/ml	0.7 µg/ml	1.0 µg/ml
		1.0 µg/ml	0.3 µg/ml	.25-.5 µg/ml
Barbiturates	.25-.5 µg/ml	2.0 µg/ml	0.5 µg/ml	100 ng/ml
		1.0 µg/ml	0.3 µg/ml	0.5 ng/ml
Methadone	0.25 µg/ml	0.5 µg/ml	0.5 µg/ml	N/A(2)
		0.3 µg/ml	0.3 µg/ml	
Benzodiazepine	1.0 µg/ml	0.7 µg/ml	0.5 µg/ml	N/A
		0.5 µg/ml	0.3 µg/ml	
Propoxyphene	0.5 µg/ml	2.0 µg/ml	1.5 µg/ml	N/A
		1.0 µg/ml	1.0 µg/ml	
Cocaine	1.0 µg/ml	1.6 µg/ml	1.5 µg/ml	100 ng/ml
Metabolite		1.0 µg/ml	1.0 µg/ml	5 ng/ml
Phencyclidine	200 ng/ml	150 ng/ml	150 ng/ml	100 ng/ml
		75 ng/ml	75 ng/ml	2 ng/ml
Cannabinoids	N/A	50 ng/ml	200 ng/ml	N/A*(3)
		20 ng/ml	100 ng/ml	

Notes: (1) Manufacturers' declared levels. First number where two are shown is recommemded cut-off level for screening, the second is the limit of sensitivity claimed.
(2) N/A = not available.
(3) N/A* = Unknown, will be available in Canada Dec 1982.

Table 2. Comparison of Screening Methods for Urine Analysis

Comparison	Test				
	TLC	Toxi-Lab	EMIT-dau	EMIT-st	Abuscreen
	Conventional Analytical Systems			Syva	Roche
Costs Initial Equipment	$5,000(1)	$3,400(1)	$12,000	$3,730	$30,000(2)
Material/assay	$2.50(3)	$12.00(3)	$13.00(4)	$26.00(4)	$10.00(5)
Single drug assays/8 hr	30-50	40-60	200-225	200-225	200-250
No. of urines/ per 8 hr (total screen)	30-50	40-60	22-25	22-25	40-50
Maximum reagent cost/8 hr	$75.00 - $125.00	$480.00 - $720.00	$286.00 - $325.00	$572.00 - $650.00	$400.00 - $500.00
Space required (6)	10	10	3	3	25
Refrigeration required	Yes	No	Yes	No	Yes
Maintenance	Minimal	Minimal	Some	Minimal	Major Equipment
Operator training	Course/TLC experience	Course/TLC experience	Minimal	Minimal	Radioisotope use
Interpretation of result	Subjective	Subjective	Objective	Objective	Objective
Sensitivity	Lowest	Lowest	High	High	Highest
Specificity	Good	Good	Low	Low	Above EMIT
Validity	All require validation by another preferably more specific method				

Notes: (1) Initial cost includes laboratory centrifuge, $1,500. Not included is fume hood.
(2) Initial cost includes gamma scintillation counter (average $25,000) and swinging bucket-type centrifuge (average $5,000).
(3) Assay is total comprehensive drug screen for all classes of drugs, standards included.
(4) Assay is total drug screen for 9 classes of drugs, calibrators included.
(5) Assay includes 5 drugs for which kits are available.
(6) Square feet, excluding ancillary equipment such as centrifuges; including fume hood, scintillation counter.

Table 3. Comparison of Confirmatory Methods for Urine Analysis

Comparison	Method		
	GLC	GC/MS	HPLC
Costs (1) Initial Equipment	$9,000 - $47,000	$87,000 - $380,000	$4,000 - $50,000
Analyses/8 hr	Variable, depends upon sample preparation time and RT of slowest component through column. May range from 8-30		
Operator Training	Extensive; skill and experience required in method setup, choice of suitable instrument parameters, etc.		
Maintenance	Extensive for all methods. Down-time may be high especially for GC/MS. Service contracts available.		
Interpretation of result	Objective for all methods with appropriate standards.		
Sensitivity	High (2)	Highest	Fairly High (2)
Specificity	Very good	Excellent Most specific	Very good
Validity	Good	Most valid	Good

Notes: (1) Range of equipment costs offered by Hewlett-Packard and Technical Marketing Associates

(2) Depends upon detector used. Low nanogram/ml levels can be achieved. For GC/MS a few hundred picograms/ml may be routine.

METHOD REFERENCES CITED BY DRUG

Amphetamines	52, 58
Barbiturates	2, 13, 75
Benzodiazepines	9, 44, 57
Cannabinoids	6, 14, 45, 55, 60, 69, 76, 77
Cocaine	47, 50, 52
LSD	12, 70, 72
Methadone	2, 33
Methaqualone	7, 8, 65
Opiates	30, 47, 52, 64
Phencyclidine	16, 35, 40, 47, 63, 74

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ANNEX A

Toxi-Lab System

TOXI-LAB...
how does it work?

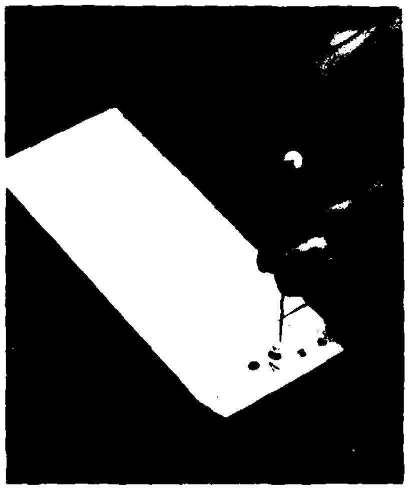
You identify unknown drugs by matching their migration and staining characteristics with the standard drugs through various stages of detection

Detect and identify analgesics, stimulants and tranquilizers with the TOXI-LAB A System

Detect and identify barbiturates and some benzodiazepines with the TOXI-LAB B System

EXTRACTION

CONCENTRATION



INOCULATION

DEVELOPMENT

Just 35 minutes from unknown to determination

TOXI-LAB A

Extraction

Pour the biological specimen (urine, serum, gastric) into the self-contained extraction tube

Tube A contains a pre-measured buffer salt (pH 9) and an organic solvent for extracting organic bases and neutral drugs

Mix and centrifuge

Concentration

Place an A blank disc into a well of the evaporation plate Transfer the plate to the warmer

Pipette the upper organic layer containing the drug into the well

Evaporate the organic layer to concentrate the drug onto the disc (approximately 5 minutes)

Inoculation

Inoculate the dried A disc into one of the center holes of the pre-standardized A chromatogram

Development

Develop the A chromatogram by placing it in a jar containing 3 ml of developing solvent and concentrated ammonium hydroxide

Pink dye markers will migrate with the solvent front When they migrate to an Rf* of 95, approximately 15 minutes, remove and briefly dry the chromatogram

*Rf = distance the drug migrates / distance the solvent front migrates

TOXI-LAB B

Extraction

Use identical procedure as TOXI-LAB A Tube B contains a pre-measured solution of buffer salt (pH 4 5) and solvents for extracting acidic and neutral drugs.

Concentration

Use identical procedure as TOXI-LAB A

Inoculation

Inoculation is identical to TOXI-LAB A

Development

Development is identical to TOXI-LAB A

Detect and identify with TOXI-LAB A

STAGE I STAGE II STAGE III STAGE IV

CHROMATOGRAM A is analyzed at four different stages. Drugs in the *unknown zone are identified* as they relate to the position and color of standard drugs in all four stages.

Stage I

Expose the A chromatogram to formaldehyde vapors for 2–3 minutes.

Dip in Mandelin's reagent (ammonium metavanadate in concentrated sulfuric acid) which causes many drugs and metabolites to appear in a spectrum of colors.

CODEINE is indicated by a dark blue spot adjacent to the dark blue codeine standard.

AMPHETAMINE is indicated by a yellowish-brown spot next to the yellowish-brown amphetamine standard.

ACETAMINOPHEN is indicated by a pale spot adjacent to the acetaminophen standard.

Stage II

Dip in water. Many drugs from Stage I will change color or disappear while new ones will appear. CODEINE is indicated by a spot which turns from blue to straw color and matches the [illegible] standard.

[illegible]

Stage III

View under long wave ultra-violet *light. Many drugs will show character*istic fluorescence or absorbance.

CODEINE is indicated by a spot which absorbs UV light adjacent to the codeine standard.

AMPHETAMINE is indicated by a blue spot adjacent to the blue amphetamine standard.

ACETAMINOPHEN is indicated by a negative or dull red spot next to the acetaminophen standard.

Stage IV

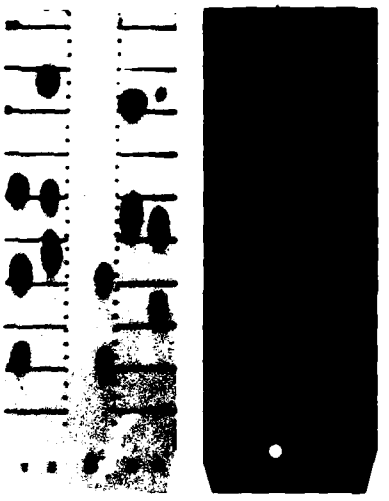
Dip in modified (iodinated) Dragendorff's reagent which stains most drugs a shade of brown.

CODEINE is indicated by a brown spot adjacent to the brown codeine standard.

AMPHETAMINE is indicated by a brown spot next to the brown amphetamine standard.

ACETAMINOPHEN is indicated by a brown spot adjacent to the brown acetaminophen standard.

Detect and identify with TOXI-LAB B



STAGE I STAGE II

CHROMATOGRAM B is analyzed at *two different stages.* Drugs in the unknown zone are identified when they match the position and color of *standard drugs* in both stages.

Stage I

Dip in a methylene chloride solution of diphenylcarbazone and air dry.

Dip successively in silver nitrate and mercuric sulfate reagents which causes barbiturates, phenytoin and glutethimide to appear as purple spots on a white background. PHENYTOIN and PHENOBARBITAL are indicated by purple grey spots adjacent to their respective standards.

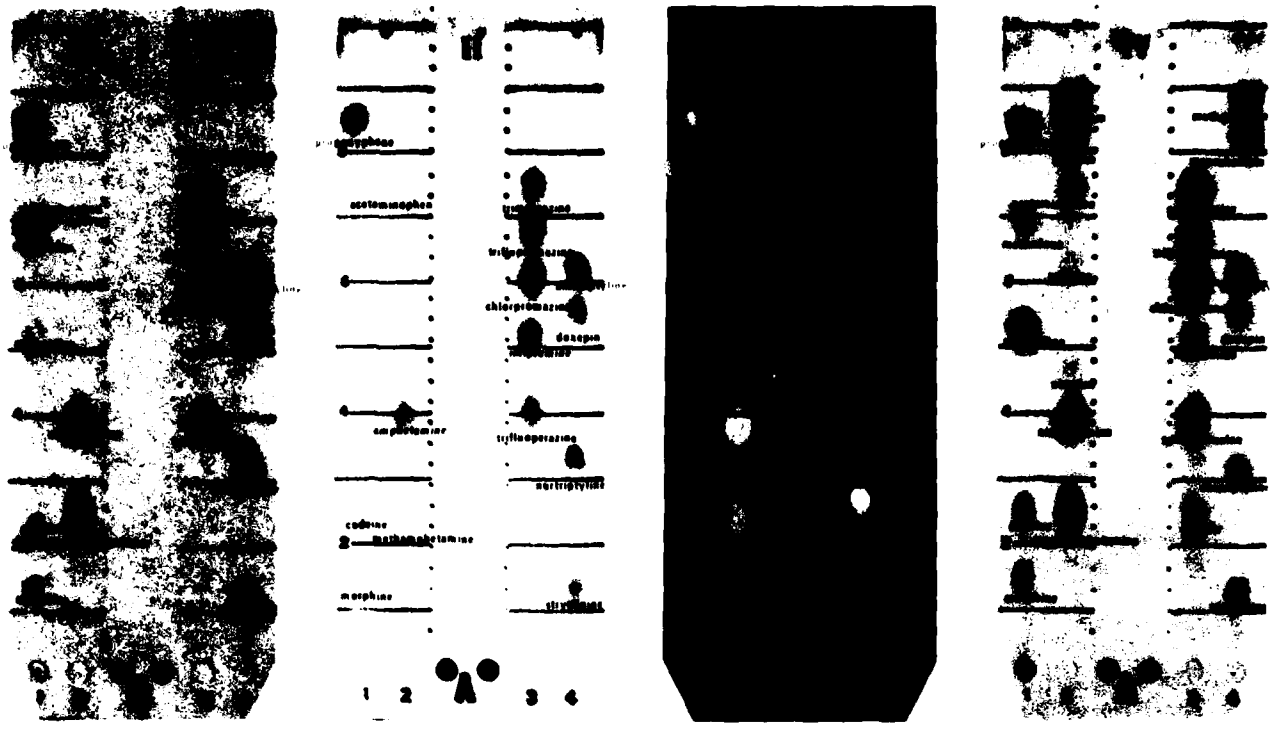
Stage II

View under long wave ultra-violet light. Barbiturates absorb the ultra violet light while certain benzodiazepines will fluoresce. PHENYTOIN and PHENOBARBITAL are indicated by absorbed spots next to their respective standards.

TOXI-LAB

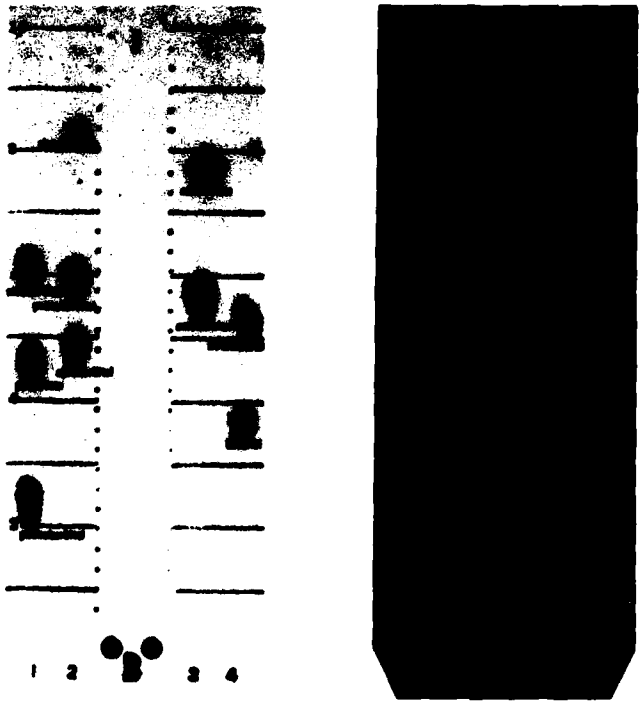
[illegible]

*COLOR DETECTION GUIDE A



* Some variation in the color and position of the drug spots is normal.

*COLOR DETECTION GUIDE B



* Some variation in the color and position of the drug spots is normal.

Analytical Systems Inc.

23162 LA CADENA DR., LAGUNA HILLS, CALIFORNIA 92653

Manufacturers of
Toxi-Lab®

(714) 770-9381
(800) 854-0277
TWX 910-595-2450
A/B: TOXI-LAB LAGH

DRUGS FREQUENTLY SUSPECT IN ABUSE AND OVERDOSE

ILLICIT DRUGS

Cocaine (methyl benzoylecgonine)
Stimulant/local anesthetic. Rapidly metabolized to benzoylecgonine and ecgonine. Free cocaine is rarely detected in urine except in cases where the dose was high. Cocaine abuse is indicated by the presence of benzoylecgonine. Methods of detection: TLC, GLC, RIA, EMIT, HI.

Heroin (diacetylmorphine)
Narcotic analgesic. Metabolized to 6-monoacetylmorphine, morphine and morphine -3-glucuronide. Hydrolysis of morphine-3-glucuronide to 'free' morphine may be indicated in abuse testing. Free morphine and possibly 6-monoacetylmorphine may be detected without hydrolysis, depending on the dose and time of specimen collection. Methods of detection: TLC, GLC, RIA, HI, EMIT. (EMIT does not differentiate morphine from other opiates, i.e., codeine.)

LSD (lysergic acid diethylamide)
Hallucinogen. Generally taken in very low doses (0.5mg). Not generally detected in biological specimens.

MDA (methylenedioxyamphetamine; 'love drug')
Hallucinogen. An analog of amphetamine. Methods of detection: TLC, GLC.

Phencyclidine (PCP; Angel Dust)
Hallucinogen; analgesic/anesthetic. Rapidly becoming the #1 drug of concern in the United States. Often combined with LSD, diphenhydramine (Benadryl), marihuana or other drugs. Methods of detection: TLC, GLC, EMIT, RIA.

Psilocybin ('magic mushrooms')
Hallucinogen. Generally taken in low doses. Not easily detected in biological specimens.

Marinuana (psychoactive agent Δ^9-tetrahydrocannabinol.)
Psychomimetic. Method of detection in biological specimens: RIA, EMIT.

PRESCRIPTION DRUGS

Barbiturates
Hypnotics. Often implicated as the causative or secondary agent in drug overdoses. Barbiturates are classified according to their 'time of onset' and 'duration': short, intermediate and long. Degree of toxicity: SHORT > INTERMEDIATE > LONG. Methods of detection: TLC, GLC, EMIT, RIA, UV spectrophotometry.

Glutethimide (Doriden)
Hypnotic. Glutethimide is sometimes abused with codeine. Methods of detection: TLC, GLC, HPLC, UV spectrophotometry.

Methaqualone (Quaalude; Sopor)
Hypnotic. Widely abused, often with alcohol and/or other drugs. Often sold illicitly. Addiction and tolerance have been noted. Methods of detection: TLC, GLC.

Benzodiazepines (Valium, Librium, Dalmane, Serax, Tranxene)
Tranquilizers. The most widely prescribed and abused drugs marketed to date. Rarely fatal when taken alone but highly toxic in combination with alcohol or other depressants. Oxazepam (Serax) is a metabolic product of most benzodiazepines. Flurazepam (Dalmane) is a hypnotic rather than a tranquilizer and is not metabolized to oxazepam. Methods of detection: TLC, GLC, EMIT.

Meprobamate (Miltown, Equinil)
Tranquilizer/muscle relaxant. Commonly prescribed and moderately abused. Overdosage is not uncommon. Methods of detection: TLC, GLC.

Phenothiazines (Thorazine, Stelazine, Mellaril, etc.)
Tranquilizers. Generally used in the management of psychoses but certain derivitives are effective antihistamines and anti-emetics. Only metabolites may be detected at therapeutic levels. Methods of detection: TLC, GLC, UV spectrophotometry, FPN screen.

Tricyclics (Elavil, Aventyl, Tofranil, Norpramin, Doxepin)
Antidepressants. Chemically related to the phenothiazines: used in the treatment of psychic depression. Abused in certain areas and often the cause of overdosage. Methods of detection: TLC, GLC, HPLC.

Morphine (opiate)
Narcotic analgesic. Not uncommonly abused by medical professionals (doctors, nurses, pharmacists, etc.) Hydrolysis of the metabolite morphine-3-glucuronide to 'free' morphine may be indicated in abuse testing. Methods of detection: TLC, EMIT, RIA, GLC.

Codeine (opiate)
Narcotic analgesic/antitussive. Generally taken in combination with other analgesics (propoxyphene) or with antihistamines. Metabolized to norcodeine and to a much lesser extent morphine. Methods of detection: TLC, EMIT, RIA, GLC.

Opiates (derived from the opium plant)
Narcotic analgesics. The most commonly prescribed opiates other than morphine and codeine are: hydromorphone (Dilaudid), hydrocodone (Dicodid) and oxycodone (Percodan). There was recently a sharp rise in abuse of these drugs with a concurrent decrease in heroin abuse. Hydrolysis is generally indicated to improve the detectability. Methods of detection: TLC, GLC, EMIT.

Meperidine (Demerol)
Synthetic narcotic analgesic. Ranks second to morphine in abuse among medical professionals. In some areas may be seen as a 'street' drug. Methods of detection: TLC, GLC, HPLC.

Methadone
Synthetic narcotic analgesic. Replacement drug for heroin addicts but often abuse Counterfeit methadone may be found on the 'street'. Methods of detection: TLC, GLC, EMIT.

Pentazocine (Talwin)
Synthetic narcotic analgesic. Abused in conjunction with tripelennamine in certain areas of the United States. Methods of detection: TLC, GLC.

Propoxyphene (Darvon)
Synthetic narcotic analgesic. Frequently abused, especially among heroin addicts. Commonly encountered in overdoses. Methods of detection: TLC, GLC.

Amphetamine (Benzedrine, Dexedrine)
Sympathomimetic amine; anorectic/CNS stimulant. Presently available in pharmaceutical preparations but is often manufactured and sold illicitly. Several areas in the United States have recently observed an increase in amphetamine (or methamphetamine) abuse. Methods of detection: TLC, GLC, EMIT.

Methamphetamine (Desoxyn, Fetamin, 'speed' 'crystal')
Sympathomimetic amine; anorectic/CNS stimulant. Available in pharmaceutical and illicit preparations. A small amount of amphetamine may be detected as a metabolite. While an increase in amphetamine abuse has been shown recently, most illicit amphetamine preparations are actually ephedrine, caffeine, phenylpropanolamine and/or diphenhydramine with amphetamine and methamphetamine being absent. Methods of detection: TLC, GLC, EMIT.

Methylphenidate (Ritalin)
Sympathomimetic amine; CNS stimulant. Used in the treatment of hyperkinetic children. Primarily excfeted as ritalinic acid and 6-oxo-ritalinic acid with approximately 1% of the dose excreted as methylphenidate. Becoming a popular drug of abuse in several regions of the United States. Methods of detection: TLC, GLC.

Phenmetrazine (Preludin)
Sympathomimetic amine; anorectic/CNS stimulant. Phenmetrazine is often used in the treatment of obesity with less stimulating effect on the central nervous system than amphetamine or methamphetamine. Abuse is not uncommon in many areas and may produce dependence similar to that of amphetamine. Methods of detection: TLC, GLC, EMIT.

Phentermine (Ionamine, Fastin)
Sympathomimetic amine; anorectic/CNS stimulant. Largely the drug of choice to replace amphetamine and methamphetamine in the treatment of obesity. While the effect on the central nervous system is considerably less than that of amphetamine, abuse of phentermine has been observed. Method of detection: TLC, GLC, EMIT.

OVER-THE-COUNTER (OTC) MEDICATIONS

Acetaminophen (Tylenol, Datril, etc.)
Analgesic. Metabolite of phenacetin (Empirin). Ingredient in most non-aspirin pain relievers. Extremely hepatotoxic in high doses. Half-life determinations are suggested for assessing toxicity. Methods of detection: TLC (screen); GLC, HPLC and spectrophotometric procedures for quantitation.

Salicylates (aspirin)
Analgesic. Common cause of overdosage either alone or combined with other drugs. Trinder's reagent for screening and quantitation.

Phenylpropanolamine (Contac, Alka-Seltzer Plus, Dexatrim, Prolamine, etc.)
Sympathomimetic amine; antihistamine/anorectic. The decongestant in most cold and allergy medications, and an appetite suppressant in most non-prescription weight control medications. Often misrepresented in illicit preparations as 'speed' or other stimulants. Methods of detection: TLC, GLC, EMIT.

Ephedrine/Pseudoephedrine (Primatene M, Bronkaid, Sudafed, etc.)
Sympathomimetic amine; antihistamine/bronchodilator. Common ingredient in many cold, allergy and asthma medications. Often misrepresented in illicit preparations as 'speed'; a common adulterant of cocaine. Methods of detection: TLC, GLC, EMIT.

Chlorpheniramine (Coricidin, Chlor-Trimeton)
Antihistamine. Ingredient found in many cold and allergy medications. Abuse has been suggested in some areas of the United States. Methods of detection: TLC, GLC.

Doxylamine (Unisom, Nyquil)
Antihistamine/mild sedative. Found in some cold medications and recently in some 'sleep-aid' medications.

Pyrilamine (Sominex, Nytol, Sleep-Eze)
Antihistamine/mild sedative. Replaced methapyriline in 1979 as the active 'sleep' ingredient. Methods of detection: TLC, GLC.

ANNEX B

Portable Urinalysis Systems Evaluation

D.M. Kouns, LCdr, USN

2-5C:DMK:LLL
15 April 1981

MEMORANDUM

From: Head, Naval Drug Screening Laboratory, Naval Regional Medical Center, Oakland CA 94627
To: Assistant Secretary of Defense (Health Affairs)
Via: (1) Commanding Officer, Naval Regional Medical Center, Oakland CA
(2) Chief, Bureau of Medicine and Surgery (MED 312)
(3) Chief of Naval Operation (OP 150D)
(4) Assistant Secretary of the Navy (MRA & L)

Subj: Portable Urinalysis Systems Evaluation

Ref: (a) OASD(HA) Memo of 19 MAY 1980; same subj; (NOTAL)
(b) My Memo 2-5C:DMK:LLL of 23 JUN 1980
(c) My ltr 2-5C:DMK:LLL of 14 MAY 1980

Encl: (1) Comparative Analysis Summary

1. Pursuant to reference (a) the evaluation of portable urinalysis systems for drug abuse detection recommended by reference (b) has been completed in accordance with the protocol recommended by reference (c). The following capsule is very respectfully submitted in advance of the annotated detailed checklists recommended in reference (c). The checklists will follow under separate cover.

2. The product summaries and scientific principles as supplied by the manufacturers are quoted as follow:

A. ROCHE DIAGNOSTICS-AGGLUTEX, a.k.a. ABUSCREEN^{R} LATEX, Latex Flocculation; LF

"The Agglutex products all utilize the same simple five step procedure which consist of:

-Add antiserum
-Add urine specimen
-Add latex antigen
-Stopper, invert once and place in heating block
-Evaluate for absense or presence of agglutination

Similiar products based on the same technology and procedure as Agglutex are routinely used in commercially marketed pregnancy tests. Roche has been an (sic) historical leader and innovator in this field. The simplicity of these products is amply demonstrated by the common use in the field by non-technical (sic) trained people.

Samples for the Agglutex assay may be tested individually or in batches up to 400 per day. This is ideally suited to the urine screening requirements of the Department of Defense.

The products are based on a latex agglutination inhibition test which integrates the specificity and sensitivity of an immunological reaction with the ease of readability and simplicity of a latex

agglutination test to produce highly accurate results.

Each individual test uses a specific antibody developed for the drug to be tested. A second reagent is provided which contains an antigen (a derivative of the drug to be tested) coupled to a latex particle. When these two reagents are mixed, the latex antigen particles agglutinate forming visable clumps. A urine sample is added to the system. In the absence of the drug being tested, this agglutination takes place.

Hense, agglutination = negative

When the drug is present in the urine, it couples to the antibody thereby inhibiting the agglutination of the latex reagent. The resulting reaction remains translucent.

Hense, lack of agglutination(a milky translucence) + positive.

B. SYVA - EMIT^{R}-st$_{IM}$; a.k.a. EMIT-SINGLE TEST

"The EMIT-st Drug Detection System consist of instrumentation, accessories and reagents for the detection of drugs of abuse in body fluids. The tests were designed as a primary screening system to detect positive samples in a largely negative population. A negative result is strong evidence that the drug in question is not present in excess of the detection limit of the assay.

The EMIT-st Assays are qualitative. They are not designed to measure quantity of a drug in a sample, but will distinguish, at least 95% of the time, a positive from a negative sample.

The EMIT-st Assays are based on a biochemical principle (enzyme immunoassay) which was developed at SYVA, and which has been used in EMIT Assays for therapeutic drug monitoring, endocrine function monitoring, and in semi-quantitative tests for drugs of abuse. The EMIT-st Drug Detection System is a simplified version of the EMIT technique.

In the performance of an EMIT-st Urine Assay, the subjects urine sample is compared against a reference solution-the Calibrator-which contains a known amount of the drug being tested for (sic). The operator uses the EMIT-st Diluter to transfer a small, fixed amount of Calibrator into one test vial, and an equal amount of the subject's urine into another vial. The vials are placed simultaneously into the EMIT-st Photometer. The EMIT-st Photometer measures and compares the reactions in the vials and within 90 seconds prints on the Result Card whether the subject's sample is positive or negative for the drug in question.

The EMIT-st Urine Assays employ a homogeneous enzyme immunoassay technique for the microanalysis of specific compounds in biological

fluids. A drug is labeled with an enzyme, and when the enzyme-labeled drug becomes bound to an antibody against the drug, the activity of the enzyme is reduced. Drug in a sample competes with the enzyme labeled drug for the antibody binding site, thereby decreasing the antibody-induced inactivation of the enzyme. Enzyme activity correlates with the concentration of drug in the sample and is measured by an absorbance change resulting from the enzyme's catalytic action on a substrate.

Each EMIT-st Assay vial contains antibodies made against a derivative of the drug in question, the coenzyme nicotinamide adenine dinucleotide (NAD), enzyme - labeled drug, enzyme substrate (glucos-b-phosphate), and Tris buffer. Reconstitution of the reagents occurs upon addition of urine and water. Drug in the sample and enzyme - labeled drug compete for binding sites on the antibodies. The amount of enzyme - labeled drug which becomes bound is dependent upon the amount of drug present in the sample. Since enzyme activity decreases upon binding, the concentration of drug in a sample can be measured in terms of enzyme activity. Active enzyme converts NAD to NADH, resulting in an absorbance change that is measured photometrically. The enzymatic activity in the sample mixture is compared against the Calibrator and the result (+ or -) is printed on the Result Card."

C. TECHNAM, INC., a.k.a. American Drug Research Institute, Inc.(ADRI)-HI-M^{R}; HI

"The test system constitutes a sealed 10 x 75mm glass tube in which is contained all of the reagents necessary to perform the test, except the specimen and diluent.

One drop of urine specimen, utilizing a disposable glass Pasteur pipette, is added to the tube, followed by six drops (or 0.3ml) of distilled or deionized water. The tube is shaken and placed in a viewing rack and observed after 90 to 120 minutes. A more detailed description of the product and system used is attached hereto as Exibit A.

A hapten molecule or molecular derivative of same, whose identification and semiquantitation is desired, is covalently linked to a carrier protein, which in turn is covalently linked to sheep red blood cells, which have been inactivated an fixed. To the hapten-micromolecule -red blood cell complex is added antiserum to the hapten at a titer, predetermined by titration, to detect a minimum amount of hapten in a body fluid. The contents, in a buffer-salts matrix, are lyophilized in 10 x 75mm glass tubes, which constitutes the prepared product that can be shipped and stored and will remain stable for a maximum of one year.

The addition of a drop of urine to the tube containing the ingredients described above will result in the removal of antibody from the hapten-macromolecule complex if the amount of hapten in the urine is at a level, predetermined by titration of the antiserum contained in

the lyophilized tube. The presence, therefore, of a hapten in a biological specimen will cause the antibody to become bound to the hapten in the specimen, resulting in a settling of cells in the tube in the form of a sharp ring. The absence of a hapten in urine or other biological specimen will result in the antibody remaining attached to the hapten-macromolecule-cell complex resulting in no reaction that can be visably seen in the bottom of the tube, because the matrix of antibody-hapten cell has prevented the cells from settling individually to form the ring. (A clear mat of cells in the tube indicates that the urine is negative, no drug is present. A positive test, equal to the predetermined concentration, will give a clear doughnut shaped ring of cells in the bottom of the tube.)

Further details of the scientific principles involved are contained in the literative attached hereto as Exihibits B, C, and D."

To summarize the foregoing, the three systems subjected to evaluation employ essentially equivalent immunochemical techniques with potentially equal sensitivities for the detection, differentiation, and identification of the various drugs of interest, but differ primarily in the techniques employed for "visualizing", "measuring", and "interpreting" the results of the assay. The ROCHE ABUSREEN LATEX system relies on a subjective technique that requires the operator to interpret degrees of agglutination, if present, and make a decision based on the visual observation of the reaction tube for the presence or absence of any agglutination particles after the 120 minutes incubation. THE SYVA EMIT-st system relies on an objective technique by employing an instrument to measure and compare the responses produced simultaneously by the urine sample and a calibrator sample, to interpret the differences, and to print the result as + or - on a form within 90 seconds. The TECHNAM (ADRI) HI-M system relies on a subjective technique that requires the operator to visually observe the reaction tube after 90 minutes for the presence of a ring of cells in the bottom of the tube and again after 120 minutes for the absence of a ring of cells or the appearance of a ring of cells that formed since the first reading.

3. The abbreviated results of my comparative evaluation of the three systems with reference to the laboratory system employing the bimodal Radioimmunoassay techniques with confirmation by Gas-Liquid Chromatography (RIA/GLC) are summarized and presented as enclosure(1). The specific details of each experiment will be documented and presented in the annotated checklists. The operators employed for this evaluation were all GS-05 level Medical Technicians (CHEMISTRY), GS-0645 series assigned to the Naval Drug Screening Laboratory. The three operators utilized during this project performed the assays on each system on a single blind basis. I personnaly prepared the various spiked samples and, in accordance, the contents and concentrations were unknown to the operators. The operators were required to read the package inserts and/or manuals for each system prior to performing the assays and to follow the protocols as written and

without additional assistance, except for clarification of terms or in the event of abberent results on the stipulated quality control requirements of each system. For the systems evaluated, no additional training was offered or required. The Known Positive Samples were obtained from the inventory of samples that had been identified by both radioimmunoassay and gas-liquid chromatography as positive and were maintained in the frozen state in our laboratory. The randomly selected urine specimens were chosen from samples that were being currently processed in the drug screening laboratory during the evaluation period. All samples processed during the evaluation were characterized by routine clinical urinalysis procedures in addition to drug urinalysis. The operators interpreted the results, where required, and recorded their data and observations on a standardized form. I transcribed the data to a master log consistent with the experimental design.

4. My personal interpretations of the results and conclusions, based on the data, observations, and experiences obtained for each of the systems subjected to evaluation and summarized in enclosure(1), as compared to the bimodal RIA/GLC reference system and expressed general and specific specifications for portable urinalysis systems for drug abuse detection are as follows:

A. General Requirements and Specifications: The three systems subjected to evaluation meet the general requirements and specifications developed for portable urinalysis systems for drug abuse detection. The three systems are designed for and have a demonstrated application to the assay of human urine for the detection, differentiation, and presumptive identification of the specific classes of drugs(and/or their major metabolites) that are commonly recognized as abused drugs or substances. The analytical techniques employed by the three systems are based on and function on specific principles that are commonly accepted by the scientific community or represent innovative combinations of accepted techniques. The three systems are based on the principles of comparative analysis such that results obtained on urine samples spiked with authentic reference materials are equivalent to results obtained on unknown urine samples that contain the drug in question. See Tables on Known Positive Samples and Cross-reacting Materials in enclosure (1). The three systems produce responses that are reasonably small and require approximately three square feet of operating space and all weigh less than thirty pounds. The ROCHE system requires electricity for the heating block and the ambient temperature must be less than 30 C and greater than 19 C. The SYVA system normally requires electricity (power options 100, 120, 220 or 240 volts), but can be battery operated, and ambient temperatures should be less than 30 C. The TECHNAM (ADRI) system requires no electricity but does require a vibration free workspace. The three systems are operable by an individual with at least a high school level of education. The package inserts provided with the systems contain sufficiently detailed information and instructions to operate the system and interpret (when required) the results. Formal "orientation" may be desired but formal training is not required. The

Subj: Portable Urinalysis Systems Evaluation

requirements for capital or investment equipment are minimal. Within the Defense supply system, items with a unit price of less than $3000.00 are classified as minor equipment and are not considered to be investment equipment.

B. Specific Requirements and Specifications: The three systems subjected to evaluation, by-in-large, failed to meet all of the rigid specific specifications developed for portable urinalysis systems for drug abuse detection. The three systems are capable of, and have the potential for expansion to become capable of detecting, differentiating, and presumptively identifying at least the following drugs (and/or major metabolites) at the indicated minimum detection (cut-off) levels and over the useful concentration range.

DRUG	MINIMUM DETECTION (Cut-off level)	USEFUL RANGE
OPIATES (as morphine glucuronide)	300 ng/ml	300-2000 ng/ml
AMPHETAMINES	1000 ng/ml	1000-5000 ng/ml
BARBITURATES (as secobarbital)	200 ng/ml	200-2000 ng/ml
PHENCYCLIDINE	25 ng/ml	25-1000 ng/ml
METHAQUALONE (metabolites)	750 ng/ml	750-3000 ng/ml
BENZOYL ECGONINE (cocaine metabolite)	750 ng/ml	750-3000 ng/ml
BENZODIAZEPINES (metabolites)	500 ng/ml	500-5000 ng/ml
CANNABINOID METABOLITES (THC-9-acid)	75 ng/ml*	75- 250 ng/ml

(* Change from original data to recognition of THC-9-acid glucoronide)

Page 1 of enclosure (1) reflects the cut-off levels for the three systems. The ROCHE system claimed greater sensitivity for the opiate (200 vice 300 ng/ml) assay and equal sensitivities for the barbiturate (200 ng/ml secobarbital) and amphetamine (1000 ng/ml) assays. The SYVA system claimed less sensitivity for the opiate (500 vice 300 ng/ml) and barbiturate (500 vice 200 ng/ml secobarbital) assays and greater sensitivity for the amphetamine (700 vice 1000 ng/ml) assay. The TECHNAM (ADRI) system provided only the opiate assay for evaluation but at two levels of sensitivity, one equal to 300 ng/ml and one with greater sensitivity (50 vice 300 ng/ml).

Page 5 of enclosure (1) presents the observed reproducibility obtained over several days on urine samples spiked at the various drug concentrations. The ROCHE opiate assay identified 100% (20/20) of the morphine samples at the 200 and 500 ng/ml levels and 95% (19/20) at its claimed cut-off 300 ng/ml. The SYVA opiate assay identified 0% (0/16) at 200 ng/ml, 43% (9/21) at 300 ng/ml and 100% (20/20) at its claimed cut-off 500 ng/ml level. The TECHNAM (ADRI) opiate assay identified 100% at its claimed levels of 50 and 300 ng/ml. The ROCHE barbiturate assay identified 0% (0/20) of the secobarbital samples at its claimed 200 ng/ml level, 25% (5/20) at 300 ng/ml, and 94% (15/16) at 500 ng/ml. The SYVA barbiturate assay identified 5% (1/20) at 200 ng/ml, 63% (10/16) at 300 ng/ml, and

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100% (20/20) at its claimed cut-off 500 ng/ml level. The ROCHE amphetamine assay identified 0% (0/10) of the amphetamine samples at 700 ng/ml and 10% (2/20) at its claimed cut-off level 1000 ng/ml level. The SYVA amphetamine assay identified 100% (20/20) at its claimed cut-off 700 ng/ml level and 100% (20/20) at the 1000 ng/ml level.

Page 6 of enclosure (1) presents the observed sensitivities of the various assays. The ROCHE opiate assay produced positive results on urine samples containing morphine at levels equal to or greater than ($\geq$) 300 ng/ml and codeine at levels equal to or greater than 200 ng/ml. The SYVA opiate assays produced positive results for morphine $\geq$ 400 ng/ml and codeine $\geq$ 100 ng/ml. The ROCHE barbiturate assay produced positive results for secobarbital $\geq$ 700 ng/ml and phenobarbital $\geq$ 5,000 ng/ml. The SYVA barbiturate assay produced positive results for secobarbital $\geq$ 300 ng/ml and phenobarbital $\geq$ 700 ng/ml. The ROCHE amphetamine assay produced positive results for amphetamine $\geq$ 1000 ng/ml and negative results for methamphetamine $\leq$ 10,000 ng/ml. The SYVA amphetamine assay produced positive results for amphetamine $\geq$ 700 ng/ml and methamphetamine $\geq$ 600 ng/ml.

The stated requirement was that the system must be able to detect the drugs at the minimum detection (cut-off) level in human urine with 95% or greater confidence. The TECHNAM (ADRI) opiate assay exceeded and met this requirement as well as the manufactures claims. The barbiturate and amphetamine assays were not available for evaluation. The ROCHE opiate assay met the requirement and the manufacturers claim, but the barbiturate and amphetamine assays failed to meet the requirements as well as failed to meet the manufacturers claims. The SYVA amphetamine assay exceeded the requirement and met the manufacturers claim. The SYVA opiate and barbiturate assays failed to meet the requirement but met the manufacturers claims.

Page 2 of enclosure (1) presents the observed reliability of the assays by the three systems on the same 528 urine samples that were processed by and compared to the Naval Drug Screening Laboratory Oakland using the bimodal RIA/GLC system. The reliability of the systems to produce the same result as the laboratory were (a) for the opiate assay 96.78% by ROCHE, 98.86% by SYVA, and 98.49% by TECHNAM (ADRI); (b) for the barbiturate assay 91.48% by ROCHE and 92.42% by SYVA ; and (c) for the amphetamine 84.47% by ROCHE and 93.90% by SYVA. Since the laboratory had a greater sensitivity for phenobarbital than the portable systems and the stated requirements, if the phenobarbital positive samples (N=70) were excluded the agreement with the laboratory on the remaining barbiturate positive samples was 90% for ROCHE and 95% for SYVA. If the known positive amphetamine population is reviewed, the agreement with the laboratory was 80.28% by ROCHE and 91.55% by SYVA.

Page 3 and 4 of enclosure (1) presents the observed false positive false negative rates of the assays by the three systems as compared to the laboratory reference system. The false positive rates on the 528

samples were (a) for the opiate assay 3.03% by ROCHE, 0.19% by SYVA, and 0.38% by TECHNAM (ADRI); (b) for the barbiturate assay 3.79% by ROCHE and 0% by SYVA; (c) for the amphetamine assay 12.88% by ROCHE and 14.96% by SYVA. For the population of known Negative samples (N = 288), the false positive rates were (a) for the opiate assay 4.17% by ROCHE, 0.35% by SYVA, and 0% by TECHNAM (ADRI); (b) for the barbiturate assay 2.43% by ROCHE and 0% by SYVA; (c) for the amphetamine assay 14.24% by ROCHE and 14.58% by SYVA. The ROCHE and SYVA amphetamine assays exceeded the requirement that the false positive rate of the samples assayed should not exceed 10%. The false negative rates on 528 samples were (a) for the opiate assay 0.19% by ROCHE, 0.95% by SYVA, and 0% by TECHNAM (ADRI); (b) for the barbiturate assay 4.74% by ROCHE and 7.58% by SYVA; (c) for the amphetamine assay 2.65% by ROCHE and 1.14% by SYVA. For the populations of known positive samples, the false negative rates were (a) for opiates (N = 80) 6.25% by ROCHE, 6.25% by SYVA, and 0% by TECHNAM (ADRI); (b) for barbiturates (N = 90) 27.78% by ROCHE and 44.44% vy SYVA; (c) for amphetamines (N = 71) 19.73% by ROCHE and 8.45% by SYVA. Excluding phenobarbital positives, the false negative rates were 10.00% by ROCHE and 5.00% by SYVA. Excluding phentermine positives from known amphetamine positive samples the false negative rates were 33.33% by ROCHE and 8.33% by SYVA. The stated requirement was that the false negative rate at the minimum detection (cut-off) level should not exceed 0.5% of all samples assayed. The TECHNAM (ADRI) opiate assay meets the requirements if the 50 ng/ml cut-off level assay is employed. Because the laboratory reference system employed lower cut-off levels, a definite conclusion with regard to the false negative rates can not be made and as indicated the test systems suffer unnecessarily by comparison.

It should be noted that the terms "NEGATIVE" and "POSITIVE" are not absolute but are defined by the minimum detection (cut-off) levels. Thus a sample that contains the drug in question at concentrations equal to or greater than the cut-off level is, by definition, positive. A sample that either contains none of the drug or contains the drug in question at concentrations less than the cut-off level is, by definition negative. The principle of any screening method is to employ a technique that is as sensitive as possible to ensure that no, or relatively low (e.g. 0.5%), false negatives are produced. Any false positive results obtained, by utilizing an extremely sensitive assay to eliminate false negatives, are eliminated by a confirmatory method. The concentration levels routinely encountered in the area of drug abuse detection are often orders of magnitude lower than those encountered in clinical settings and incident related testing and the sensitivity levels of the screening techniques and specifications to define positive and negative must be concordant. The onus resides with the manufacturer to established cut-off levels for the assays that are lower than the stated minimum detection (cut-off) levels to assure a confidence level of 95% for positives and a low false negative rate at the minimum detection (cut-off) level. The "apparently" high 10% false positive rate allows for a reduction in specificity to gain the sensitivity desired for a

screening procedure. The specifications of a program should not be modified to meet the requirements of a particular system, but rather the system should be modified to meet the requirements of the program. Pragmaticism pertains to the scientific evolution of causes and effect , and philosophically to the notion that concepts are predictions of facts to be found and consequences to result, should specified action be taken. If the intent of an onsite, portable system is to produce low false positives rates, then a higher rate of false negatives will occur and a certain percentage of individuals who need assistance will escape detection. If the intent is to produce low false negative rates, then a higher rate of false positives will occur, but the rights of that certain percentage of those individuals will be protected by a confirmation procedure. Under such a system, negative results are indeed strong indications for the absence of use or abuse.

Pages 7, 8, and 9 of enclosure (1) present the results of an experiment designed to show the effect that salt (Sodium Chloride, NaCl) when added to the urine sample, would have on the three systems evaluated. The addition of salt at levels of 5g/100ml had no effect on the assays evaluated.

The stated requirement that the total operator time for the preparation of a single assay should not exceed five minutes was met by all systems. However, the total time required to obtain a single assay could be of concern in some applications. The ROCHE and TECHNAM (ADRI) assays require two hours to obtain a result. The ROCHE system protocol recommends storage of the bulk reagents in a refrigerator and requires that the reagents be at room temperatore prior to analysis. The time period required to bring the reagent to room temperature will vary with the ambient temperature but could add 30 to 45 minutes to the total assay time. With both the ROCHE system and the TECHNAM (ADRI) system, security of the assay must be maintained during the two hour incubation period for the following reasons. If the reaction tubes of the ROCHE system are inverted any time during or after the two hour incubation period, all samples appear POSITIVE, including the controls, and a repeat assay would require an additional two hour period. If the reaction tubes of the TECHNAM (ADRI) system are shaken during or after the two hour incubation period, all samples appear NEGATIVE and a repeat assay would require an additional two hour period. The SYVA assays require two minutes to obtain a result. Security of the assay is not required. If the reaction tubes or the result card is removed during the assay an error message is printed on the result card. A repeat assay requires an additional two minutes.

The stated requirement that the system must be expandable and capable of adding a new drug assay within nine months of a specific request can be met by all three systems.

5. An area of concern that was expressed by all services in the concept study report, as well as by the customers of the laboratories, involves the effect of interferring or cross-reacting substances on the assays and results obtained by the various analytical systems. Page 11 of enclosure (1) and the attached tables present data to address

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this area.

ROCHE provided the following statment under limitations of the assay : "It should also be noted that high concentrations of protein in urine may reduce the degree of agglutination and urine samples with high specific gravity may affect the sensitivity of the cut-off point." I reviewed the routine urinalysis data on the samples processed by the four systems and discovered that this affect is indeed observed in actual practice. The data is presented on the log sheet attached to page 11 of enclosure (1). On samples that contained 2^+ or 3^+ protein a false positive barbiturate and/or opiate result was obtained by the ROCHE system, but not by the SYVA or TECHNAM (ADRI) systems. This phenomenon could be of concern in the military population where strenuous excercise is routine. Proteinuria is not an uncommon clinical finding in runners for example.

To test the reactivity and specificity of the assays to a broad spectrum of substances or compounds for potential cross-reactivity and/or interference, I prepared 200 urine samples, from a known negative urine pool, that contained one of 100 different drugs at a concentration of either 2 micrograms/milliliter or 30 micrograms/milliliter. The 200 samples were processed, on a single blind basis, by each of the drug assays of the three systems under evaluation. The results are presented in alphabetical order in the tables attached to page 11 of enclosure (1).

The ROCHE opiate assay produced positive results for the following compounds: ALPHAPRODINE (30mcg/ml), CODEINE, CYCLAZOCINE (30mcg/ml), DEXTROMETHORPHAN (30mcg/ml), DIDRATE, DIHYDROHYDROXYCODEINONE, ETHYLMORPHINE, HYDROCODONE, MEPERIDINE (30mcg/ml), METHADONE (30mcg/ml), MORPHINE, NALORPHINE, PCP PYRROLIDINE ANALOG (30mcg/ml), TCP PYRROLIDINE ANALOG (30mcg/ml), and THEBAINE. The SYVA opiate assay produced positive results for the following compounds: ALPHAPRODINE (30mcg/ml), CODEINE, DIDRATE, DIHYDROHYDROXYCODEINONE, ETHYLMORPHINE, HYDROCODOINE, MORPHINE, NALORPHINE (30mcg/ml), TCP PYRROLIDINE ANALOG (30mcg/ml), and THEBAINE. The TECHNAM (ADRI) opiate assay produced positive results with the following compounds: CODEINE, DIDRATE, ETHYLMORPHINE, HYDROCODONE, MORPHINE, NALOPHENE (30mcg/ml), and THEBAINE.

The ROCHE barbiturate assay produced positive results with the following compounds: ALLYLCYCLOPENTENYLBARBITAL, ALLYLISOBUTYLBARBITAL, ALPHENAL, AMOBARBITAL, APROBARBITAL, BARBITAL, BUTABARBITAL, BUTETHAL, DIALLYLBARBITAL, MEPHOBARBITAL (30mcg/ml), PENTOBARBITAL, PHENOBARBITAL, SECOBARBITAL, and THIAMYLAL. The SYVA barbiturate assay produced positive results with the following compounds: ALLYLCYCLOPENTENYL-BARBITAL, ALLYLISOBUTYLBARBITAL, ALPHENAL, AMOBARBITAL, APROBARBITAL, BARBITAL (30mcg/ml) BUTABARBITAL, BUTETHAL, DIALLYLBARBITAL, HEXOBARBITAL (30mcg/ml), MEPHOBARBITAL, PENTOBARBITAL, PHENOBARBITAL, PRIMIDONE (30mcg/ml), SECOBARBITAL, and THIAMYLAL (30mcg/ml).

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The ROCHE amphetamine assay produced positive results with the following compounds: AMPHETAMINE, METHAMPHETAMINE (30mcg/ml), and PHENTERMINE (30mcg/ml). The SYVA amphetamine assay produced positive results with the following compounds: AMPHETAMINE, BENZPHETAMINE, EPHERIN, METHAMPHETAMINE, PHENAZOCINE (30mcg/ml), PHENTERMINE, and PHENYLPROPANOLAMINE.

The following compounds produced negative results by all the assays, except when noted above: ACETAMINOPHEN, ACETYLSALICYLIC ACID, 4-AMINOANTIPYRINE, AMINOPHYLLINE, AMITRIPTYLINE, BENZOYL ECGONINE, CAFFEINE, CARBROMAL, CLONAZEPAM, CHLORAL HYDRATE, CHLORDIAZEPOXIDE, CHLOROQUINE, CHLOROTHIAZIDE, CHLORPHENIRAMINE, CHLORPROMAZINE, COCAINE, N-DESMETHYL-DIAZEPAM, DESTROPROPOXYPHENE, DIAZEPAM, DIMETHYL-METHYLSUCCINIMIDE, PHENYTOIN, DYPHYLLINE, ECGONINE, ETHINAMATE, FLURAZEPAM, GLUTETHIMIDE, IMINOSTILBENE, LIDOCAINE, MEPROBAMATE, MESCALINE, METHAPYRILENE, METHAQUALONE and METABOLITES, METHPRYLON, 4-METHYLPRIMIDONE, NITRAZEPAM, NICOTINE, NOSCAPINE, OXAZEPAM, PAPAVERINE, PENTAZOCINE, PHENACETIN, PHENCYCLIDINE, PCP N-ETHYL-ANALOG, PCP 4-DYDROXY METABOLITE, PCP MORPHOLINE ANALOG, PHENDIMETRAZINE, PHENYLEPHRINE, PROBENECID, PROCAINE, QUINACRINE, QUINIDINE, SCOPOLAMINE, SULFAPYRIDINE, TETRACAINE, and ZOXAZOLAMINE.

6. Page 12 of enclosure (1) provides the comparative cost data for the systems evaluated.

7. If I personnaly had to choose one system as the portable, on-site urinalysis system for the rapid, presumptive detection of drugs of abuse to be used as an adjunct to the present DOD laboratory system, based on the accumulated data, observations, and experiences obtained during this evaluation, I would, with certain reservations, recommend the SYVA EMIT-single test system. The nature of the SYVA system to employ an objective technique to place the onus on an instrument to measure the reaction, interpret the data, and print a positive or negative result on a form within a 90 second period of time instead of relying on the operator's individual judgement and/or integrity; the absence of a requirement for security over a long assay time period; and the engineered diagnostics and error codes of the system, are factors that influence my viewpoint. My reservations concern the cost of the system and the sensitivity of the barbiturate assay for the most commonly detected barbiturate phenobarbital. The subjective nature of interpreting the results of the ROCHE system; the protein interference; the requirements for refrigerated storage of the bulk reagents and security during a long assay time; and the ambient temperature requirements of the assay are factors that influence my viewpoint. In a stable, secure laboratory environment with operators experienced in interpreting degrees of agglutination, and if the amphetamine assay was responsive to methamphetamine, the ROCHE latex system could be an acceptable substitute for the Radioimmunoassay procedures. The effects of vibration and shock on the development of the TECHNAM (ADRI) assay result; the requirement for security during the assay time period; the availability of only one assay for evaluation; the subjective nature of the interpretation; the reliance on

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the operator's judgement and/or integrity; and the long assay time period are factors that influence my view point. In a stable, secure laboratory environment, and if the other assays are as equally sensitive as the opiate assay, the TECHNAM (ADRI) system would be an ideal replacement for the Radioimmunoassay procedures presently employed in the drug testing laboratories.

9. Reference (a) stated"...it is now necessary to examine all the portable test kits on the market to determine which, if any, can satisfy the military services' requirements for portable equipment. The Navy is requested to assume that responsibility. Specifically, the Navy is requested to examine all existing portable kits for drug abuse detection (there are estimated to be eight) and to conduct such technical tests as are deemed necessary to determine if any are suitable for use within the military services." This advanced capsule of the report consisting of the annotated checklists, to be submitted under separate cover, is the culmination of the requested examinations and technical testing. It is my opinion that the SYVA EMIT-Single Test system is suitable for use within the military services as a portable kit for drug abuse detection.

Very respectfully,

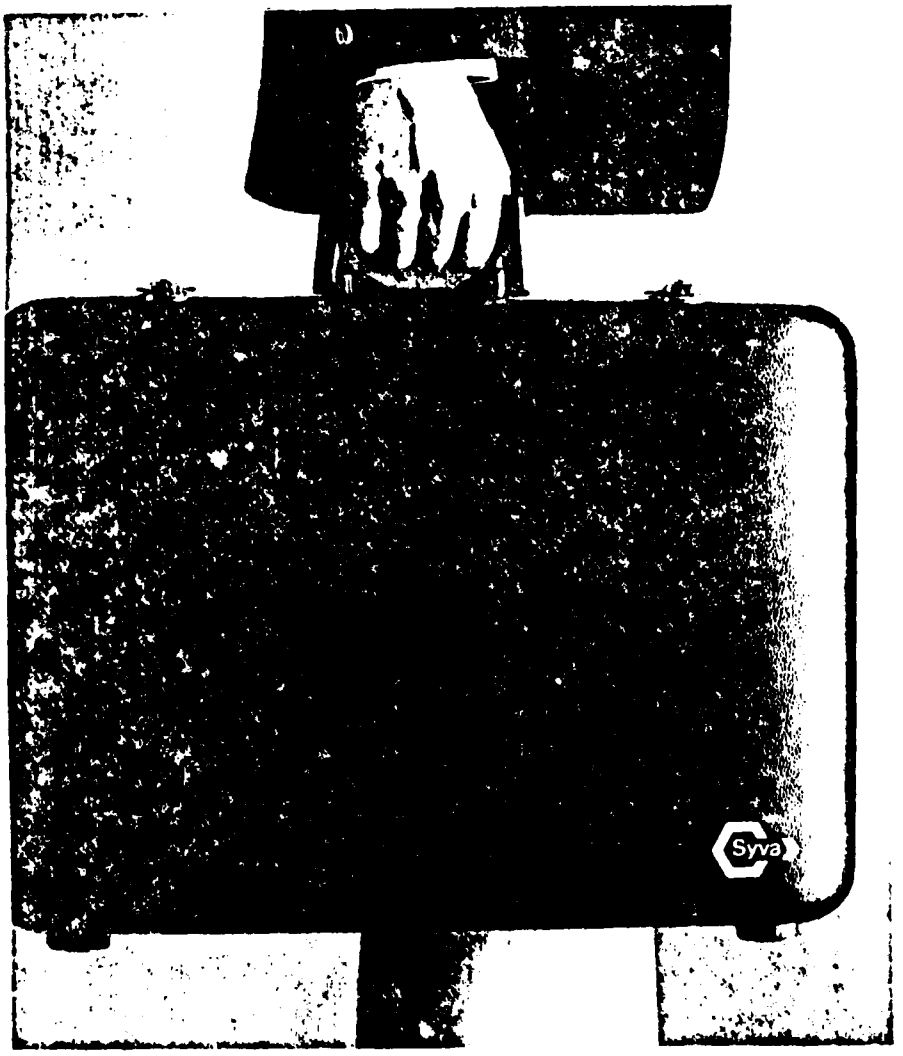
DAVID M. KOUNS
Lieutenant Commander,MSC,U.S. Navy

Copy to:
CHLABSER NRMC OAKLAND
COL TRAHAN
COL LATHROP
COL MANDERS

ANNEX C

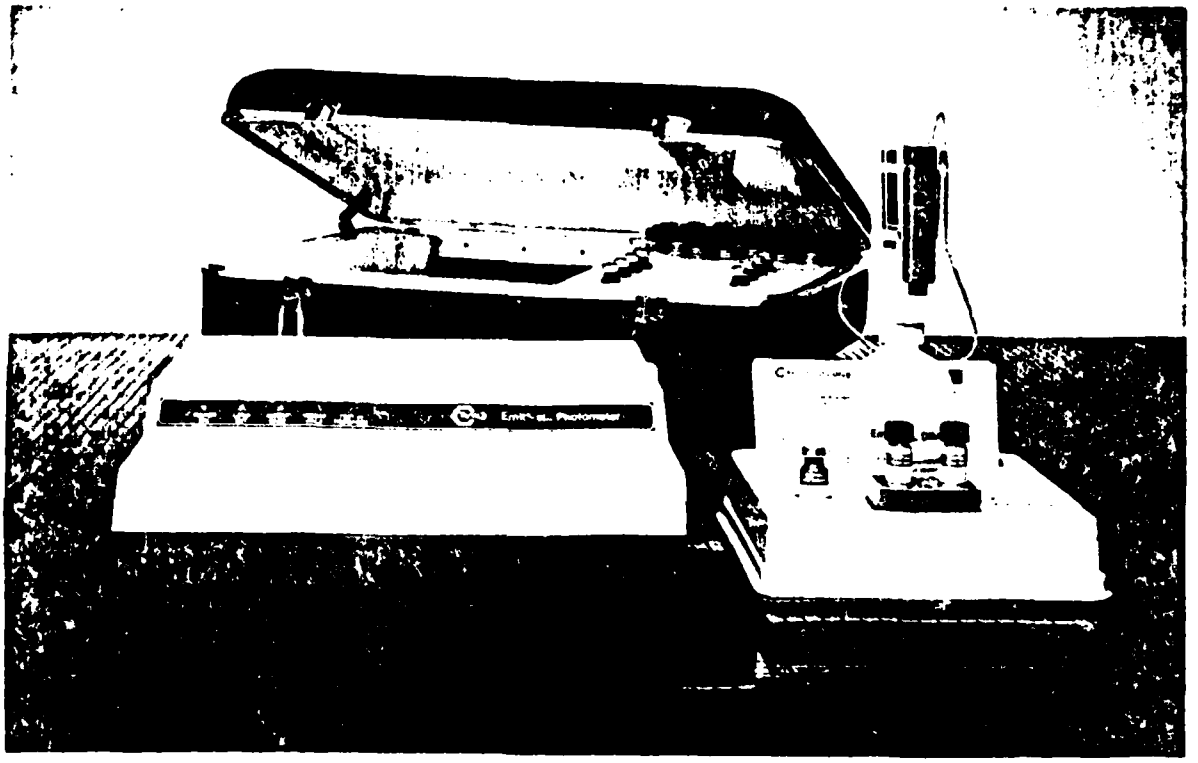
EMIT-st Drug Detection System

ON-SITE DRUG DETECTION HAS NEVER BEEN THIS EASY



New EMIT-ST™

Stat
toxicology
system



ON-SITE DRUG DETECTION HAS NEVER BEEN THIS EASY

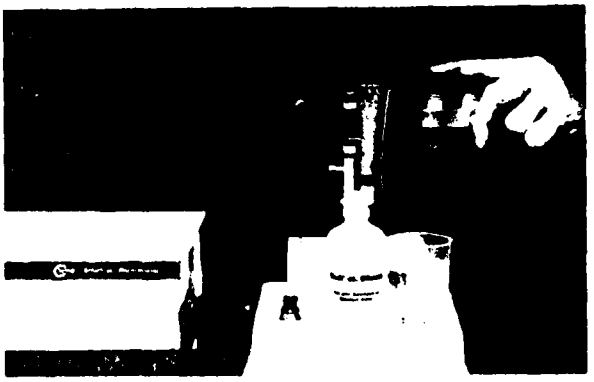
1 Use diluter to pick up a pre-measured amount of calibrator.

2 Press plunger to release calibrator into left test vial.

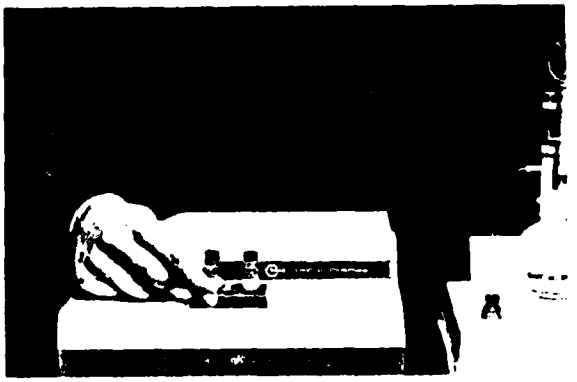
3 With diluter, pick up a pre-measured amount of urine sample.

4 Dispense this sample into right test vial.

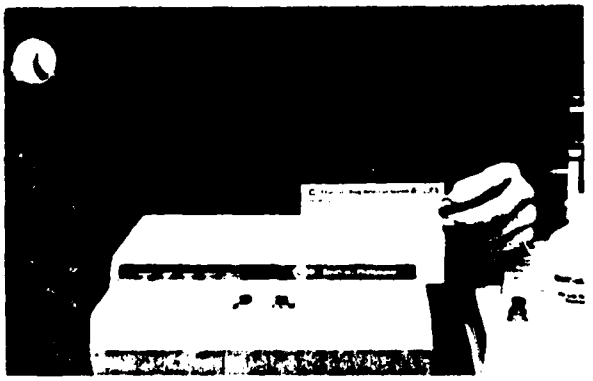
[illegible] variables that affect urinary [illegible] pattern, amount ingested, route of [illegible] [illegible] drug in question [illegible] of intoxication. Results should be confirmed by [illegible] interview or by an alternate, equally sensitive analytical method when loss of rights, or other corrective action is contemplated.



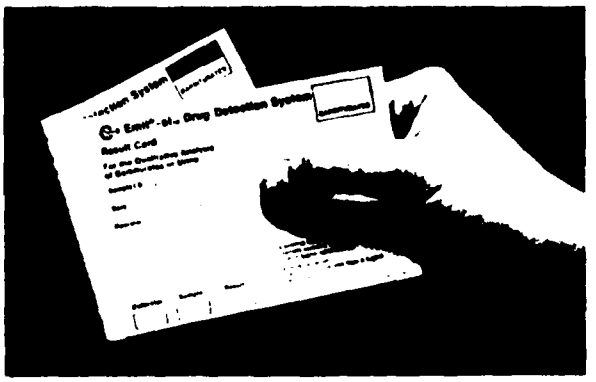
5 Shake vial holder for 15 seconds.



6 Insert vial holder into instrument well.



7 Insert test card into slot on instrument



8 In 90 seconds the instrument "sees" if the subject's sample has more or less drug than the calibrator and a positive (+) or negative (−) result will be automatically printed on test card

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